A Project Proposal

on

Evidence based evaluation of Medicinal Plants used for treating Polycystic Ovary Syndrome

Submitted to

University Grants Commission
(Major Research Project)

(MRP-MAJOR-BOTA-2013-22123)
F.No. – 43-121/2014 (SR)

from

Dr. Sunita Shailajan
Principal Investigator

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Co-Investigator

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NEW DELHI – 110 002

ASSESSMENT CERTIFICATE

(To be submitted with the proposal)

It is certified that the proposal entitled, "Evidence Based Evaluation of Medicinal plants used for treating Polycystic Ovary Syndrome" F. No. - 43-121/2014(SR) by Professor Sunita Shailajan, Deptt. of Botany has been assessed by the Reviewer committee consisting the following members for submission to the University Grants Commission, New Delhi for financial support under the scheme of Major Research Project

Details of Expert Committee:

- **Dr. (Smt.) Savita Kulkarni**
  Scientific Officer (G),
  Head, Immunoassay Development & Tuberculosis Immunology section
  Radiation Medicine Centre (B.A.R.C)
  c/o, Tata Memorial Hospital Annexe,
  Parel, Mumbai, Maharashtra- 400012.

- **Dr. B.B. Sharma**
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The proposal is as per the guidelines.

(REGISTRAR/ PRINCIPAL)

(Seal)

ANNEXURE IX

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PROFORMA FOR SUBMISSION OF INFORMATION AT THE TIME OF SENDING THE FINAL REPORT OF THE WORK DONE ON THE PROJECT

1	**TITLE OF THE PROJECT:**	Evidence based evaluation of Medicinal Plants used for treating Polycystic Ovary Syndrome
2	**NAME OF THE PRINCIPAL INVESTIGATOR:**	Dr. Sunita Shailajan
3	**ADDRESS OF THE PRINCIPAL INVESTIGATOR:**	Office: F-13, Herbal Research Lab, Ramnarain Ruia Autonomous College, Matunga (E), Mumbai- 400 019.
4	**NAME AND ADDRESS OF THE INSTITUTION:**	Ramnarain Ruia Autonomous College, Matunga (E), Mumbai- 400 019.
5	**UGC APPROVAL LETTER NO. AND DATE:**	F. NO. - 43-121/2014(SR) ; FD Diary No.- 2026 Dated: 8th July 2015
6	**DATE OF IMPLEMENTATION:**	5th September 2015
7	**TENURE OF THE PROJECT:**	3 years, from 1st July 2015-30th June 2018
8	**TOTAL GRANT ALLOCATED:**	15,49,000/- (Fifteen Lakhs Forty Nine Thousand only)
9	**TOTAL GRANT RECEIVED:**	13,45,240/- (Thirteen Lakhs Forty Five Thousand Two Hundred and Forty only)
10	**FINAL EXPENDITURE:**	14,42,723/- (Fourteen Lakhs Forty Two Thousand Seven Hundred and Twenty Three only)
11	**TITLE OF THE PROJECT:**	Evidence based evaluation of Medicinal Plants used for treating Polycystic Ovary Syndrome

12. OBJECTIVES OF THE PROJECT:

A) Overall:

a) To develop quality control methods for the use of *Mimosa pudica* (whole plant) and *Symplocos racemosa* (stem bark) in order to get consistent quality of extracts.

b) To standardize *Mimosa pudica* and *Symplocos racemosa* extracts for their use in the management of PCOS and suggest a possible use of these two plants in the ayurvedic formulations.

c) To establish the efficacy of extracts of *Mimosa pudica* and *Symplocos racemosa* in the management of PCOS.

B) Specific:

a) Collection and authentication of *Mimosa pudica* and *Symplocos racemosa* from various geographical regions of India.

b) To develop and evaluate quality control parameters for establishing the stability and content uniformity of the selected plant materials as per standard pharmacopeial guidelines (IP, API and HP)

c) Preparation of enriched extracts of selected plant materials using solvents of different polarities and finally to use the solvent for extraction with maximum extractive value.

d) Standardization of phytochemical fingerprints of plant extracts using chromatographic techniques like HPTLC and HPLC.

e) Identification and quantitation of bioactive marker (s) from the plant extracts and to validate the developed method as per ICH guidelines.

f) The chemical profiles of the enriched extracts may be confirmed for bioactive(s) using LC-MS-MS technique.

g) To evaluate the safety of enriched extracts by conducting acute toxicity study in mice as per OECD guidelines.

h) To evaluate efficacy of enriched extracts in the management of PCOS using Letrozole (1.0 mg/kg) induced rat model and to compare the results with treatment of Clomiphene citrate (1.0 mg/kg).

i) To evaluate bioavailability of the enriched extracts individually using controlled pharmacokinetics studies on rat/rabbit using HPTLC/HPLC.

j) To publish scientific data in suitable journal.

13. WHETHER THE OBJECTIVES WERE ACHIEVED: Yes the objectives were achieved as per the timelines given.

14. ACHIEVEMENTS FROM THE PROJECT:

Papers published

- Application of modern RP-HPLC technique for the quantitation of betulinic acid from traditional drug *Symplocos racemosa* Roxb. Journal of Applied Pharmaceutical Sciences (Sr, No. 19203 in UGC list of Journals).

- Impact of geographical variation on rutin content from *Mimosa pudica* L. using HPTLC technique. International Journal of Research in Pharmacy and Chemistry (Journal No. 48705 in UGC list of Journals).

Papers presented at conferences:

- Comparative HPTLC and HPLC estimation of Betulinic acid from *Mimosa pudica* collected from various geographical regions of India.
- Comparative estimation of Betulin from *Symplocos racemosa* collected in different seasons using validated chromatographic techniques.
- Pharmacokinetic profile of Betulinic acid from *Symplocos racemosa* Roxb. in Wistar rats using validated RP-HPLC method.
- Marker based standardization of an Ayurvedic plant: *Mimosa pudica* L.

15. SUMMARY OF THE FINDINGS (IN 500 WORDS):

The current research work was on use of whole plant of *Mimosa pudica* Linn. and bark of *Symplocos racemosa* Roxb. in the management of Poly Cystic Ovary Syndrome which has become more prevalent problem in today's era. *M. pudica* and *S. racemosa* have been standardized in terms of quality control parameters. The values obtained for proximate analysis parameters were found to be within the permissible limits. Further phytochemical profile of the plants was carried out prior to chromatography. *M. pudica* showed maximum amount of quaternary alkaloids and bark of *S. racemosa* showed maximum amount of basic extracts- most alakloids. Chromatographic fingerprint was developed and therapeutically potent biomarker, betulinic acid from *Symplocos racemosa* bark and gallic acid from *Mimosa pudica* whole plant was quantitated using validated HPTLC and HPLC techniques. The plants were further subjected for its safety. Before conducting safety studies the study protocols were approved by Institutional Animal Ethics Committee members as per CPCSEA guidelines. The extracts were then subjected to safety evaluation in albino Swiss mice following OECD guidelines no. 420. It was found to be safe upto a maximum dose of 2000 mg/kg body weight and no mortality or clinical toxicological symptoms were observed. The individual plants were evaluated for their therapeutic potency where animals were divided into groups *viz*, normal control, letrozole control, natural recovery, *Mimosa pudica* and *Symplocos racemosa* each group was induced with letrozole for 21 days. After the treatment period of 21 days, blood samples were drawn to determine testosterone, estrogen, progesterone and total cholesterol. The animals of each group were then given different treatments for recovery with the individual plants for 15 days post letrozole induction. After the study period, they were sacrificed biochemical parameters, ovary and uterine weights of all groups were determined. Histopathological examination of the ovary was done under Light and Electron microscope. Statistical evaluation of data was done using ANOVA followed by Student's t test and Dunnett's test. Gallic acid has been used for .The bioavailability study of the extracts of *Smplocos racemosa* and *Mimosa pudica* was estimated in the rat model by quantifying the bioactive marker gallic acid in plasma using HPLC technique and parameters like C_{max}, $T_{max,}$ K_{el}, AUC, $t_{1/2}$ etc which were analysed using WinNolin.

16. CONTRIBUTION TO THE SOCIETY (GIVE DETAILS):

- *Mimosa pudica* and *Symplocos racemosa* are traditionally important Ayurvedic plants which have been used in the treatment of various female reproductive disorders. The quality control parameters adopted in this research work to standardize *M. pudica* and *S. racemosa* can be used by various manufacturers of formulations containing *M. pudica* and *S. racemosa*. Chromatographic technique used to develop fingerprints can be used to authenticate and identify the plant material and thus can prevent the use of adulterants. The detection of bio active marker from plants can be used to isolate and identify the therapeutic potency from other biological matrices.

17. WHETHER ANY PH.D. ENROLLED/PRODUCED OUT OF THE PROJECT:

This project work is a part of the PhD thesis work of the Junior Research Fellow working on the project.

Name of the Junior Research Fellow: Ms. Suhina Bhosale

Subject: PhD in Bioanalytical Sciences

PhD thesis title: Validation of *Myrica esculenta* Buch. – Ham. Ex D. Don and *Mimosa pudica* L. in the management of Polycystic Ovary Syndrome.

18. NO. OF PUBLICATIONS OUT OF THE PROJECT:

- Sunita Shailajan, Sasikumar Menon, Dipti Singh, Gauri Swar, Suhina Bhosale Application of modern RP-HPLC technique for the quantitation of betulinic acid from traditional drug *Symplocos racemosa* Roxb. Journal of Applied Pharmaceutical Sciences (Sr, No. 19203 in UGC list of Journals).
- Sunita Shailajan, Sasikumar Menon, Suhina Bhosale
  Impact of geographical variation on rutin content from *Mimosa pudica* L. using HPTLC technique. International Journal of Research in Pharmacy and Chemistry (Journal No. 48705 in UGC list of Journals).

PRINCIPAL INVESTIGATOR

REGISTRAR/PRINCIPAL
(Seal)

CO-INVESTIGATOR

ANNEXURE IX

Timelines				
Phase	**Duration**	**Milestone**	**Status**	
First year	**2 months**	**Collection and authentication of the plants.**	**Completed**	**Report submitted**
	3 months	**Evaluation of quality control parameters for short listed plants.**	**Completed**	
	3 months	**Preparation and standardization of enriched extracts**	**Completed**	
	4 months	**Phytochemical profiling of plant extracts using HPTLC and HPLC method.**	**Completed**	
Second year	**2 months**	**Quantitation of bioactive marker and its validation**	**Completed**	
	9 months	**Safety and efficacy (dose dependent) evaluation of enriched extracts on animal system**	**Completed**	
	1 month	**Documentation of data for publication and report preparation**	**Completed**	
Third year	**4 months**	**Efficacy (effective dose) evaluation of enriched extracts on animal system**	**Completed**	**Report attached**
	4 months	**Establishing bioavailability of enriched extracts individually in rat/ rabbit system**	**Completed**	
	4 months	**Documentation of data for publication and report preparation**	**2 papers published 1st and 2nd year report submitted**	

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Objectives achieved in first year

- Collection and authentication of *Mimosa pudica* and *Symplocos racemosa* from various geographical regions of India.
- To develop and evaluate quality control parameters for establishing the stability and content uniformity of the selected plant materials as per standard pharmacopeial guidelines (IP, API and HP)
- Preparation of enriched extracts of selected plant materials using solvents of different polarities and finally to use the solvent for extraction with maximum extractive value.
- Standardization of phytochemical fingerprints of plant extracts using chromatographic techniques like HPTLC and HPLC.

Objectives achieved in second year

- Identification and quantitation of bioactive marker (s) from the plant extracts and to validate the developed method as per ICH guidelines.
- The chemical profiles of the enriched extracts may be confirmed for bioactive(s) using LC-MS-MS technique.
- To evaluate the safety of enriched extracts by conducting acute toxicity study in mice as per OECD guidelines.

Objectives achieved in third year

- To evaluate efficacy of enriched extracts in the management of PCOS using Letrozole (1.0 mg/kg) induced rat model and to compare the results with treatment of Clomiphene citrate (1.0 mg/kg).
- To evaluate bioavailability of the enriched extracts individually using controlled pharmacokinetics studies on rat/rabbit using HPTLC/HPLC.
- To publish scientific data in suitable journal.

Final report

(ANNEXURE IX)

1. Introduction to Ayurveda

Traditional systems of medicines have been developed in various parts of the world from time immemorial. A systematic shape has been given to them in many ancient civilisation and culture. According to Charaka, Ayurveda has always been in existence and there had always been people who understood it in their own way. Some of the traditional systems are based on rational and sound fundamental principles and some others have only an empirical base. Some of these traditional systems did not survive and have become subjects of history of medicine like the Greek medicine and the Egyptian medicine. Some others like the traditional systems of medicine of India and China are not only surviving, but also fully progressing as healing powers with state patronage. Ayurveda, Siddha, Unani, *Emchi* (Tibetan) and *Prakruti chikitsa* (Naturopathy) are the various traditional systems of medicine still prevalent in India. Besides, several types of folk medicines are prevalent in different tribal areas of India. They have a rich tradition of use of plants, minerals and animal products having therapeutic utility (Singh and Dey, 2005; Vaidya, 1999;Ayurveda Encyclopedia, 1998;). Due to these unique features of Ayurveda, some of the ayurvedic plants have been used for the treatment of various ailments related to different parts of the body and also disorders caused by hormonal imbalances related to the female reproductive system.

2. Disorders of Female Reproductive System

The female reproductive system consists of two ovaries, two oviducts (also called as uterine or fallopian tubes), a uterus, a vagina, external genitalia and two mammary glands (breasts). The disorders of the system involve the improper functioning of any of the organs as mentioned above, major ones being the ovary and the uterus. Some of the disorders are as follows, Premenstrual Syndrome, Premenstrual dysphoric disorder (PMDD), ovarian cysts, Endometriosis, Uterine Leiomyomas or Fibroids, Pelvic Inflammatory Disease (PID), Menorrhagia. Modern medicine treatment is also available for the above mentioned disorders

PMS can be treated by giving antidepressants as Fluoxetine, Sertaline etc., nonsteroidal anti-inflammatory drugs as Diclofenac, Ketoprofen, Ibuprofen, Meclofenamate, Naproxen and Mefenamic acid (Frank J, 2005; Speroff *et al*, 1999; Yonkers *et al*, 1997; Steiner *et al*, 1995). People with PMDD are relieved by treatment with antidepressants Fluoxetine, Sertraline and Paroxetine (Frank J, 2005; Pearlstein and Stone, 1994).

Ovarian cysts can be treated with clomiphene citrate, or intramuscular injection of progesterone, with oral contraceptives, etc. They can also be treated by surgery (Frank J, 2005). Polycystic ovary syndrome can be treated by Clomiphene citrate, Metformin, Spironolactone, Eflornithine and by surgical treatment (Lebinger and Tessa, 2007; Khan and Klachko, 2006; Frank J, 2005; Barclay, 2004; Ganie *et al*, 2004; Seli and Duleba, 2002; Velazquez *et al*, 1997).

Menorrhagia can be treated by administering nonsteroidal anti-inflammatory drugs, tranexamic acid, by anti-inflammatory drugs, or by oral contraceptives. (Hurskainen *et al*, 2007; Speroff *et al*, 1999; Bonnar and Sheppard, 1997; Rosenfield, 1996; Van Eijkeren *et al*, 1992; Milsom *et al*, 1991; Nelson and Rybo, 1971 ;).

3. Treatment by Ayurveda / Herbal medicine

- Premenstrual syndrome can be treated with plants like Black cohosh, St. John's wort, evening primrose oil, Ginkgo biloba and chasteberry (Frank, 2005).
- Polycystic ovary syndrome can be treated by evening primrose oil, false unicorn root, blue cohosh, Chinese herbs, such as gui zhi fu ling wan. It can also be treated by alternative treatment like supplement of vitamins and homeopathy (Glenville, 2007; Fallon , 2006).
- Meryton tablet is another formulation that is used for treating irregular periods, PMS and other symptoms of menstrual disorders. It has *Saraca indica, Symplocos racemosa, Tinospora cordifolia* and *Tribulus terrestris* as ingredients.
- Ashokarishta is another herbal formulation which has herbs like *Saraca indica, Zingiber officinale, Piper longum, Eugenia caryophyllus, Cinnamom tamala, Elettari cardomomum, Plumbago zeylanica, Embelia ribes, Piper chaba, Juniperus communis, Terminalia chebula, Terminalia belerica, Emblica officinalis, Vandas roxburghii, Glycyrrhiza glabra, Anacycus pyrethrum, Abutilon indicum*, etc. It is effective for the treatment of gynecological conditions like menorrhagia, leucorrhoea and dysmenorrhoea. It is also used in leucorrhoea, haematuria, and other female conditions.

4. Polycystic Ovary syndrome (PCOS)

In 1935, Stein and Leventhal first described a symptom complex associated with anovulation (Speroff et al, 1994; Danowski, 1962; Keettel et al, 1957). They were first to recognize an association between the presence of polycystic ovaries and signs of hirsutism and amenorrhea (e.g., oligomenorrhea, obesity). Women diagnosed with Stein-Leventhal syndrome underwent successful wedge resection of the ovaries, their menstrual cycles became regular, and they were able to conceive. As a consequence, a primary ovarian defect was detected and the disorder came to be known as polycystic ovarian disease. Further biochemical, clinical, and endocrinology studies revealed an array of underlying abnormalities; hence, the condition is now referred to as "Polycystic Ovary syndrome" (PCOS). It is also called as "Stein-Leventhal syndrome" (Hughesdon, 1982; Stein and Leventhal, 1935). It is a condition characterized by the accumulation of numerous cysts (fluid-filled sacs) in the ovaries associated with high male hormone levels, chronic anovulation (absent ovulation) and other metabolic disturbances (Fallon, 2006).

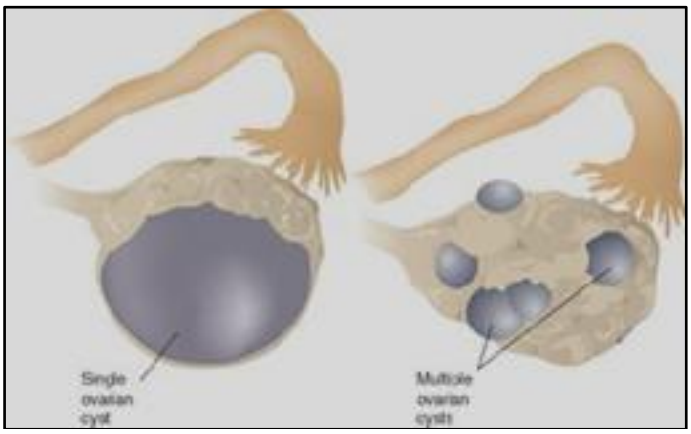


Figure 1: Polycystic ovary

5. Causes of PCOS

While the exact cause of PCOS is unknown, but, it is believed that the tendency to develop the syndrome may be inherited. The interaction of hyper insulinemia and hyper androgenism is believed to play a role in chronic anovulation in susceptible women (Fallon, 2006). The inciting event which causes the disease is not known, but, PCOS involves interactions of hormonal abnormalities, some of which are self-perpetuating (Marx and Mehta, 2003).The different clinical features seen in patients with PCOS are: Menstrual abnormalities, Hyperandrogenism, Infertility, Obesity, Diabetes mellitus, Physical Hirsutism. Other features observed in Women with PCOS is they suffer from cardiovascular diseases (Duleba et al, 2006; Wild, 2002; Wild et al, 2000; Dahlgreen et al, 1992)Polycystic ovary syndrome is also associated with increased oxidative stress and elevated markers of systemic inflammation such as C-reactive protein (Duleba et al, 2006; Kelly et al, 2001; Sabuncu et al, 2001).

6. Medical Management of PCOS

Medical management is aimed at the treatment of metabolic derangements, anovulation, hirsutism, and menstrual irregularity. Modern medicines like Metformin (Glucophage), Antiandrogens, such as spironolactone (Aldactone), are effective for hirsutism. Spironolactone 50-100 mg twice daily is an effective primary therapy for hirsutism. (Barclay 2004; Marx and Mehta, 2003; Evans and Burke, 1986; Cumming *et al*, 1982). Eflornithine (Marx and Mehta, 2003; Med.Lett Drugs Ther, 2000). Other drugs used are Ethinyl estradiol (Estinyl) (Khan and Klachko, 2006; Physician Desk Reference, 2005). Clomiphene citrate (Hughes *et al*, 2003; Shanbag and Bhattacharya, 2003; Kousta *et al*, 1997; Gysler *et al*, 1982; Gorlitsky *et al*, 1978; Garcia *et al*, 1977). Simvastatin (statins) (Duleba *et al*, 2006), Surgical treatment of PCOS may be performed if drug treatment fails, but it is not common.

Thus, PCOS is a heterogenous group of disorders, each responding differently to individual treatments. No single drug can be used to treat PCOS. Several drugs cater to the different symptoms of PCOS. The drugs used to treat PCOS have a lot of side-effects.

7. Experimental work

The current project work deals with the standardisation of medicinal plants; *Mimosa pudica* Linn. and *Symplocos racemosa* Roxb. for the treatment of polycystic ovary syndrome which is one of the disorders of female reproductive system. As per literature, these two plants have been researched a lot for their chemical constituents, physiological properties and pathophysiological properties.

Available Literature on *Mimosa pudica and Symplocos racemosa*:

A lot of chemical constituents like mimosine (alkaloid), stigmasterol, leucoanthocyanidin, D-xylose and D-glucuronic acid, norepinephrine, D-pinitol, linoleic acid, oleic acid, palmitic acid, stearic acid, β-sitosterol, crocetin dimethyl ester are reported to be present in Mimosa pudica Linn. (Singh and Jabri, 2001; Yadava and Yadav, 2001). *Mimosa pudica* Linn. has larvicidal property (Sharma and Wattal, 1979). It is used to treat menorrhagia and leucorrhoea (Sharma et al, 2001; Vaidya and Sheth, 1986; Hemadri and Rao, 1983). The plant has diuretic (Pillai et al, 1978) and hyperglycemic activity (Amalraj and Ignacimuthu, 2002). Similarly *Symplocos racemosa* Roxb. has chemical constituents like flavanol, glucosides like symplocosides, symposide, leucopelargonidin 3-glucoside, ellagic acid, flavonol glycoside like rhamnetin 3-digalactoside, triterpenoids like, 19 α-hydroxyarjunolic

acid-3, 28-O-bis-β-glucopyranosides, 19 α-hydroasiatic acid-3, betulin, Oleanolic acid, β-sitosterol, α-amyrin, apart from these chemical constituents bark mainly contains alkaloids like loturine, isoloturine and harmane, (Bhusnar et al, 2014). A number of pharmacological properties have been reported for the bark of the plant which includes wound healing activity, anti-fibrinolytic activity, skin topical agents, preventing skin aging and skin whitening agents. In indigenous system of medicine it has been used as anti cancer agent. The bark of *Symplocos racemosa* is also used traditionally by the local and tribal people of South India for the treatment of diarrhoea, dysentery, oxytocis, amoebicide, anticancer, conjunctivitis, ophthalmia, bleeding gums, menorrhagia and other uterine disorders (Vijayabaskaran et al, 2010).

8. Plant material in the present study

Plants under investigation in the present work are *Mimosa pudica* and *Symplocos racemosa*. The whole plant of *Mimosa pudica* was collected from different regions of Ratnagiri, Maharashtra. The stem bark of *Symplocos racemosa* was collected from different geographical regions of India. The respective plant parts under investigation were cleared off from all visible foreign matter by washing. The collected plant material was kept on blotting paper to allow the water to drain off. Following this, the plant material were wrapped in news paper and then kept for shade drying. Herbaria were prepared of each plant species and sent for authentication to Agharkar Research Institute, Pune.

8.1 Authentication of Plant material

It is mandatory to authenticate the plant for its identity by an authority recognized by the Central Government, like NBRI/NISCOM/BSI in India or by any other institution recognized by Central or State Government. Herbariums of the plants are dispatched to the centers for authentication. This is done for all plant raw materials and relevant certificates are achieved.

In the present work, the whole plant of *Mimosa pudica* and stem bark of *Symplocos racemosa* were authenticated from Agharkar research Institute, Pune.

8.2 Identification of the plant species

According to WHO guidelines, the first stage in assuring quality, safety and efficacy of herbal medicines is identification of the plant raw material by its species or botanical verification by the currently accepted Latin Binomial name and synonyms.

The first stage of a phytotherapeutic investigation is the selection of the herbal material to be tested (Evans, 2008). There are three different types of approaches to select medicinal species in the pharmacological investigation: i) at random – when plant – selection criterion is not used (i.e. they are randomly investigated, according to the species availability); ii) chemotaxonomic – when species are selected according to the occurrence of a certain chemical class of a substance which belongs to a genus or family; iii) ethnopharmacologic – when plants are selected according to the therapeutic usage exposed by a certain ethnic group (Gupta, 2010; Kapoor, 2005).

In the present work, the plant species are identified from each other on the basis of Bentham and Hooker classification (Sambamurty, 2005). *Mimosa pudica* Linn. and *Symplocos racemosa* Roxb. were identified and classified according to the classification system laid down by Bentham and Hooker (Warming, 1932; Rendle, 1925).

8.2.1 *Mimosa pudica* Linn.

8.2.1.1 Classification

Division	-	Spermatophyta (seed-bearing plant)
Sub-division	-	Angiosperms (flowering plants)
Class	-	Dicotyledons (seed with two cotyledons)
Subclass	-	Polypetalae (petals are free)
Series	-	Calyciflorae (flowers perigynous or epigynous)
Order	-	Rosales (flowers are bisexual)
Family	-	Leguminosae (fruit is a legume)
Subfamily	-	Mimosae (leaves bipinnate, flowers regular)
Genus	-	*Mimosa*
Species	-	*pudica*

8.2.1.2 Vernacular names

English- Touch-me-not,
Hindi- Lajjavanti,
Lajvanti, Lajalu,
Bengali- Lajjabati,
Gujarati- Lajalu,
Kanada- Lajja,
Malayalam- Tottavadi,
Marathi- Lajalu

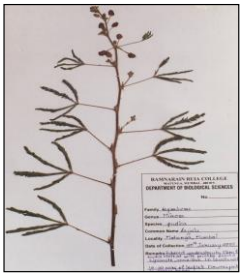
8.2.1.3 Habitat

Mimosa pudica Linn. is a prostate or suberect shrub. Stems and rachis are clothed with prickles. Leaves are bipinnate and sensitive to touch. Pinnae are 2-4, digitatively arranged, with 10-20 pairs of leaflets. Flowers are pinkish and arranged in globose heads. Pods are small, flat, straw coloured, with many bristles and with 3-5 seeds (Sharma *et al*, 2001; Cooke, 1958). It is a native of tropical America (Brazil), naturalized nearly throughout the tropical and sub-tropical parts of India. It is found in Sub-Himalayan tracts, West Bengal, damper districts of Bihar and Orissa, Maharashtra and hot moist localities of southern states (Sharma *et al*, 2001).

Figure 2: Habitat of *Mimosa pudica*

Figure 3: Leaves of *Mimosa pudica*

Figure 4: Inflorescence of *Mimosa pudica*



Authentication No. ARI 10-75 of 8/06/2010

Figure 5: Herbarium of *Mimosa pudica*

8.2.2 *Symplocos racemosa* Roxb.

8.2.2.1 Classification

Division	-	Spermatophyta (seed-bearing plant)
Sub-division	-	Angiosperms (flowering plants)
Class	-	Dicotyledons (seed with two cotyledons)
Subclass	-	Gamopetalae (petals are fused)
Series	-	Heteromerae (flowers perigynous or epigynous)
Order	-	Ebenales (flowers are bisexual, pentamerous, ovary superior)
Family	-	Symplocaceae
Genus	-	*Symplocos*
Species	-	*racemosa*

8.2.2.2 Vernacular names

Sanskrit - Lodhra,
English - Symplocos bark,
Hindi - Lodh,
Marathi - Lodhra, Lodh,
Gujarati - Lodhar, Lodar,
Tamil - Vellilathi, Vellilothra

8.2.2.3 Habitat

A small, evergreen tree, up to 6 – 8.5 m tall; bark dark grey, rough. Leaves 10-15 x 4 - 6 cm, elliptic, serrate, rarely entire, glabrous and dark green above, pubescent beneath**.** Spikes rusty – pubescent, 4-6 times as long as petiole. Bracts hirsute, the middle one ovate, the lateral lanceolate, all of the same length and equaling or overtopping the calyx.Flowers are white, fading yellow, in simple axillary racemes. Calyx glabrous, lobes rounded, slightly longer than the tube. Corolla twice as long as the calyx, 5-6-partite. Calyx stamens about 80, scarcely longer than the corolla, ovary puberulous. Fruit (drupe) is oblong, 1-1.3 cm long, purplish black when ripe, crowned with persistent calyx (Khare, 2004 and Sharma *et al*, 2002). It is found in the plains and lower hills throughout North and East India, ascending in the Himalayas up to an elevation of 1400 m, Bengal, Assam and Chota Nagpur (Kapoor 2005 and Sharma *et al*, 2002).

Figure 6: Twig of *Symplocos racemosa* showing fruits

Figure 7: Stem bark of *Symplocos racemosa*

Figure 7: Habitat of *Symplocos racemosa*

Authentication No.ARI 10-173 of 24/12/2010

Figure 8: Herbarium of *Symplocos racemosa*

9. Quality Control Methods

Quality control is a term that refers to the processes involved in maintaining the quality of the raw materials and the manufactured product. Regardless of the form of herbal preparation, some degree of quality control should exist and to ensure their quality, they need to be standardised. Standardisation is the system that ensures that the raw materials contain the correct substance in the correct amount and will induce its therapeutic effect. Parameters such as identification, authentication, estimation of extractive values, ash values, chromatographic profiles, etc. are used for the standardisation of the herbal plants. The estimation of the above parameters also ensures that the purity of raw materials is maintained. The proportion of different constituents in the plant raw materials used in the medicinal preparations may vary according to the place of collection, the season and different environmental conditions. Variability is also seen in the herbal medicines due to the use of several plants formulated together in the same preparation. Thus, a quality control test for the entire preparation can ensure the quality of the preparation (Prajapati *et al*, 2003).

The therapeutic activities of plants depend not only on the use of proper plant ingredient and its concentration, but also on the presence of required quality and nature of secondary metabolites in the raw material. The availability of secondary metabolites, in turn, depends on environmental and other factors like time and season of collection, procedures of drying and storage and geographical variations. Unless the plants are standardised, the efficiency of the herbal product is affected and thereby it can lead to failures in the therapy (Prajapati *et al*, 2003).

In the present work, the extractive values as alcohol soluble extract and water soluble extract and ash values as total ash and acid insoluble ash of *Mimosa pudica* Linn. and *Symplocos racemosa* Roxb. were calculated and the values were found to be in accordance with the limits mentioned in the literature (ICMR, 2011 and 2006).The different phytoconstituents as Fats & waxes, Phenolics & terpenoids, Alkaloids, Quarternary alkaloids & N-oxides of *Mimosa pudica* Linn. and *Symplocos racemosa* Roxb. were extracted by using Soxhlet apparatus and separated using High Performance Thin Layer Chromatography (HPTLC) The fingerprint of *Mimosa pudica* Linn. and *Symplocos racemosa* Roxb. was developed using HPTLC.

9.1 Proximate analysis

Evaluation of the crude drugs is of great importance for the phytochemical industry. It involves the determination of identity, purity and quality. Purity depends upon the absence of foreign organic matter whether organic or inorganic, while quality refers to the concentration of the active constituents in the drugs. It has been emphasized to ensure the quality of the medicinal plant products by using modern control techniques and to apply suitable standards before using these materials in manufacturing processes. The crude drug should be tested for the following tests as per the United States Pharmacopoeia and Indian Herbal Pharmacopoeia. Some of the tests are:

9.1.1 Foreign organic matter:

Medicinal plant material should be entirely free from visible signs of contamination that is moulds, insects and other animal contamination, including animal excreta and organic debris. It is seldom possible to obtain marketed plant materials that are entirely free from some form of organic matter (WHO, 1998). Any soil, stones, sand,

dust and other foreign organic matter must be removed before medicinal plant materials and cut or ground for manufacturing processes.

Observations

It is observed that the percentage of foreign organic matter in *Mimosa pudica* whole plant powder is 0.34 ±0.04%and *Symplocos racemosa* stem bark powder is 0.11 ± 0.02%. The percentage of foreign organic matter in *Mimosa pudica* whole plant powder is found to be higher.

Table 1: Percentage of Foreign Organic Matter in *Mimosa pudica* whole plant and *Symplocos racemosa* stem bark

Sample	% Mean ± SD	Prescribed limits
Mimosa pudica	0.34 ±0.04	NMT 2% (Rao, 1999)
Symplocos racemosa	0.11 ± 0.02	NMT 1% (Sharma *et al*, 2002; Rao, 2001)

9.1.2 Extractable matter

Extractive values are useful for the evaluation of a crude drug, gives idea about the nature of the chemical constituents present in the crud drug and are useful for the estimation of specific constituents, soluble in that solvent used for extraction. The method determines the amount of active constituents extracted with solvents from given amount of medicinal plant material (Mukherjee, 2002). Here according to the method given in the Indian Herbal Pharmacopoeia ethanol and water were used as solvents to determine the extractable matter.

Observations

It is observed that the percentage of water extractable matter in *Mimosa pudica* whole plant powder is 23.62 ± 2.21% and *Symplocos racemosa* stem bark powder is 21.04 ± 0.75%.

Table 2: Percentage of Water extractable Matter in *Mimosa pudica* whole plant and *Symplocos racemosa* stem bark

Sample	%Mean ± SD	Prescribed limits
Mimosa pudica	23.62 ± 2.21	NLT 9% (Rao, 1999)
Symplocos racemosa	21.04 ± 0.75	NLT 15% (Sharma *et al*, 2002; Rao, 2001)

Mimosa pudica whole plant powder is 13.69 ± 1.09%and *Symplocos racemosa* stem bark powder is 13.18 ± 0.79%.

Table 3: Percentage of Ethanol extractable Matter in *Mimosa pudica* whole plant and *Symplocos racemosa* stem bark

Sample	%Mean ± SD	Prescribed limits
Mimosa pudica	13.69 ± 1.09	NLT 9% (Rao, 1999)
Symplocos racemosa	13.18 ± 0.79	NLT 9% (Sharma *et al*, 2002; Rao, 2001)

9.1.3 Ash content

Ash values are used to determine the quantity and purity of a crude drug. Ash contains inorganic radicals like phosphates, carbonates and silicates of sodium, potassium, magnesium and calcium etc. sometimes inorganic variables like calcium oxalate, silica, and carbonate content of the crude drug affects total ash value. Such variables

are then removed by treating with acid (as they are soluble in hydrochloric acid) and then acid insoluble ash value is obtained (Mukherjee, 2002).
The ash remaining following the ignition of medicinal plant material is determined by three different methods, which measure

1) Total ash
2) Acid-insoluble ash
3) Water soluble ash

9.1.3.1 Total ash

The total ash method is designed to measure the total amount of material remaining after ignition. This includes both 'physiological ash' that is derived from the plant tissue itself and 'non-physiological ash' that is the residue of the extraneous matter (e.g. sand and soil) adhering to the plant surface. Ashing involves an oxidation of the components of the product. A high ash value is indicative of contamination, substitution, adulteration or carelessness in preparing the crude drug for marketing. Total ash is designed to measure the total amount of material produced after complete incineration of the ground drug at as low temperature as possible (at about 450°C) to remove all the carbons. At higher temperature, the alkali chloride may be volatile and may be lost by this process. The total ash usually consists of carbonates, phosphates, silicates and silica which include both physiological and non-physiological ash (Mukherjee, 2002). As per the Indian Pharmacopoeia the total ash is determined by the following procedure.

Observations

It is observed that the percentage of total ash content in *Mimosa pudica* whole plant powder is 6.24 ± 0.22%and *Symplocos racemosa* stem bark powder is 7.12 ± 0.23%.

Table 4: Total ash content for *Mimosa pudica* whole plant and *Symplocos racemosa* stem bark

Sample	%Mean ± SD	Prescribed limits
Mimosa pudica	6.24 ± 0.22	NMT 10% (Rao, 1999)
Symplocos racemosa	7.12 ± 0.23	NMT 12% (Sharma *et al*, 2002; Rao, 2001)

9.1.3.2 Acid insoluble ash

Acid insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid and igniting the remaining insoluble matter. This measures the amount of silica present especially as sand and siliceous earth.
Acid insoluble ash is frequently necessary to evaluate the crude drugs, which indicate the residue obtained after treating the total ash with dilute hydrochloric acid and weighing the residue. This ash value particularly indicates the contamination of siliceous material e.g. earth and sand; comparison of this with the total ash value of the same sample will differentiate between contaminating materials and variations of the natural ash of the drug. The value for this acid insoluble ash varies from 0.5% (agar) to as much as 12% (Hysosyamus) (Mukherjee, 2002).

Observations

It is observed that the percentage of acid insoluble ash content in *Mimosa pudica* whole plant powder is 1.99 ± 0.52%and *Symplocos racemosa* stem bark powder is 0.40 ± 0.05%.

Table 5: Acid insoluble ash content for *Mimosa pudica* whole plant and *Symplocos racemosa* stem bark

Sample	%Mean ± SD	Prescribed limits
Mimosa pudica	1.99 ± 0.52	NMT 5% (Rao, 1999)
Symplocos racemosa	0.40 ± 0.05	NMT 1% (Sharma *et al*, 2002; Rao, 2001)

9.1.3.3 Water soluble ash

Water soluble ash is the difference between the total ash and the residue after the treatment of the total ash with water. It is that part of the total ash content which is soluble in water. It is a good indicator of either previous extraction of the water soluble salts in the drug or incorrect preparation (Mukherjee, 2002). As per the Indian Pharmacopoeia the water soluble ash is determined by the following procedure.

Observations

It is observed that the percentage of water soluble ash content in *Mimosa pudica* whole plant powder is 0.42 ± 0.13%and *Symplocos racemosa* stem bark powder is 0.20 ± 0.03.

Table 6: Water soluble ash content for *Mimosa pudica* whole plant and *Symplocos racemosa* stem bark

Sample	%Mean ± SD	Prescribed limits	Suggested limits
Mimosa pudica	0.42 ± 0.13	Not specified in literature	NMT 0.93%
Symplocos racemosa	0.20 ± 0.03	Not specified in literature	NMT 0.31%

9.1.4 Loss on drying

The Loss on Drying Test is designed to measure the amount of water in a sample when the sample is dried under specified conditions.

Observations

It is observed that the percentage of loss on drying of *Mimosa pudica* whole plant powder is 11.51 ± 0.87% and *Symplocos racemosa* stem bark powder is 6.96 ± 0.34%.

Table 7: Percentage of loss on drying for Mimosa *pudica* whole plant and *Symplocos racemosa* stem bark

Sample	%Mean±SD	Prescribed limits	Suggested limits
Mimosa pudica	11.51 ± 0.87	Not specified in literature	NMT 15.01%
Symplocos racemosa	6.96 ± 0.34	Not specified in literature	NMT 8.31%

10. Phytochemical Analysis

When investigating the complete phytochemical profile of a given plant species, fractionation of a crude extract is desirable in order to separate the main classes of constituent from each other prior to chromatography. In the current work, different phytoconstituents like Fats & waxes, Phenolics & terpenoids, Alkaloids, Quarternary alkaloids & N-oxides present in *Mimosa pudica* Linn. and *Symplocos racemosa Roxb.* were purified involving solvents of varying polarity and separated using High

performance thin layer chromatography (HPTLC) by using the method given by (Harborne, 1998).

Table 8: Percentage of phytochemical Constituents of *Mimosa pudica* Linn. and *Symplocos racemosa* Roxb.

Phytochemical Constituents	**% Extract* (*Mimosa pudica*)**	**% Extract* (*Symplocos racemosa*)**
Neutral extract - Fats & waxes	0.283 ± 0.027	0.214 ± 0.154
Moderately polar extract-Terpenoids and Phenolics	2.336 ± 0.322	1.286 ± 0.074
Basic extract-Most alkaloids	2.051 ± 0.176	**40.550 ± 0.221**
Polar extract-Quaternary alkaloids and N-oxides	**10.731 ± 0.256**	2.4480 ± 0.116
Fibers	84.252 ± 0.123	41.240 ± 0.036

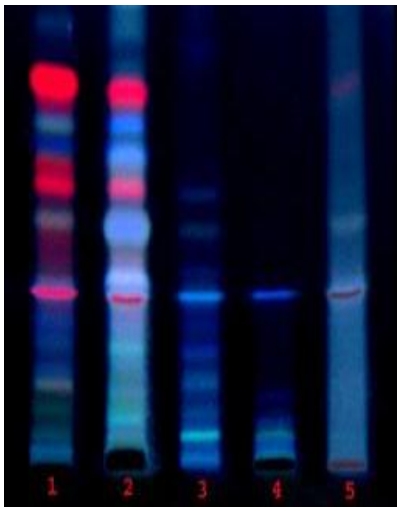
* Each result is expressed as Mean ± Standard deviation of three readings.

The profile showed that *Mimosa pudica* Linn. has maximum amount of quaternary alkaloids and N-oxides, similarly stem bark of *Symplocos racemosa* Roxb. showed maximum amount of Basic extract-most alkaloids.

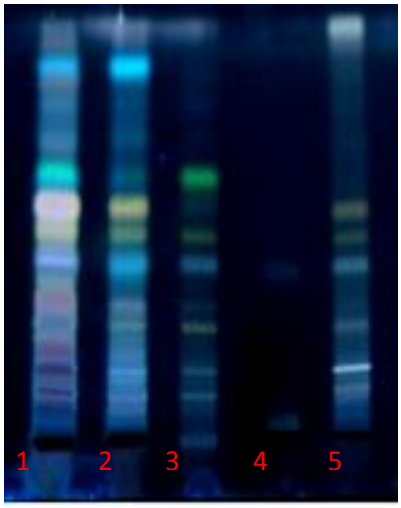
Table 9: Optimized chromatographic HPTLC conditions for phytochemical constituents of stem bark of *Symplocos racemosa* Roxb and whole plant of *Mimosa pudica* Linn.

Parameters	***Mimosa pudica***	***Symplocos racemosa***
Stationary Phase	Merck Silica gel 60 F_{254} HPTLC precoated plates	
Mode of separation	Normal phase	
Mobile phase	Dichloromethane: Toluene: Methanol: Glacial acetic acid, 7.5:2:0.4:0.1, (v/v/v/v)	Toluene: Methanol, 8:1, (v/v)
Development chamber	Camag twin trough chamber	
Chamber saturation	25 minutes	30 minutes
Sample applicator	Camag Linomat IV	
Band width	7 mm	6 mm
Space between the bands	15mm	10mm
Rate of sample application	10 secs/µL	15 secs/µL
Development distance	80 mm	85 mm
Derivatisation	10% Methanolic Sulphuric acid reagent	
Densitometric scanner	Camag Scanner II WinCats 3 Software	
Lamp, wavelength	Mercury,366nm	

Figure 9: Chromatographic overlay of *Mimosa pudica* Linn. and *Symplocos racemosa* Roxb. alongwith its phytoconstituents



Track 1- *Mimosa pudica* extract
Track 2- Phenolics and terpenoids
Track 3- Alkaloids
Track 4- Quarternary alkaloids & N-oxides
Track 5- Fats & Waxes



Track 1– Terpenoids and Phenolics
Track 2 – Alkaloids
Track 3 – *Symplocos racemosa* extract
Track 4 – Quaternary alkaloids and N-oxides
Track 5– Fats & waxes

11. High Performance Thin Layer Chromatography and High Performance Liquid Chromatography

A fingerprint of a herbal medicine is a chromatogram here representing all the detectable chemical components present in the extract and being separated as much as possible so as to identify and characterize that herbal medicine with the help of the fingerprint, the authentication and identification of a herbal Medicine can be reliably conducted even if the quality and quantity of the constituents are unknown. When we deal with chromatographic fingerprints of Herbal medicines from different sources, we can do similarity evaluation or pattern recognition for quality control purposes (Cheng-Jian Xu et al, 2006). A marker can be defined as a chemical entity, in the plant material, which may or may not be chemically defined and serves as a characteristic fingerprint for that plant. In other ways through various analytical techniques like TLC, HPLC and HPTLC we can visualize the presence of this compound in the plant and also quantify it to ascertain the limits. A biomarker on the other hand is a group of chemical compounds which are in addition to being unique for that plant material also correlate with biological efficacy. Batch to batch variations start from the collection of raw material itself in the absence of any reference standards for proper identification, and multiply during storage and further processing. So the need arises to lay standards by which the right material is selected and incorporated into the formulation.

Chemical marker for herbal standardization- Fingerprinting in essence means establishing a characteristic chemical pattern for the plant material or its cut or fraction or extract. It is important to understand that a plant extract consists of established classes of chemical compounds. These include very interesting and useful classes of compounds like alkaloids, flavonoids, coumarins, terpenoids, anthocyanins, etc, and we can utilize these secondary metabolites for the identification of plant material as our knowledge of chemistry has advanced sufficiently and through sophisticated analytical techniques we can measure these compounds qualitatively and quantitatively. (Shrikumar and Ravi, 2007). Thus chromatography offers the best method for recording the fingerprint which can be produced anywhere, provided the conditions are maintained (Govindarajan and Vijaykumar, 2005; Liang, 2004).

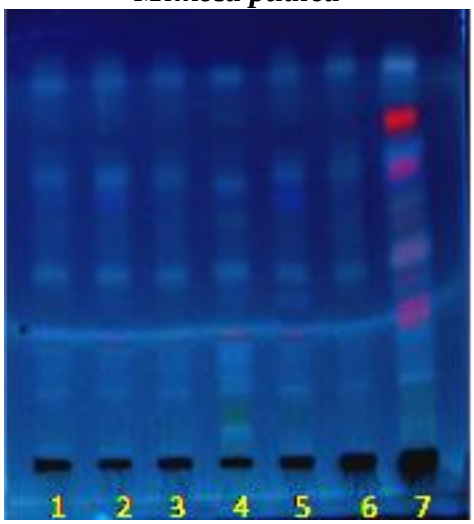
Table 10: Optimized chromatographic HPTLC fingerprint conditions for whole plant of *Mimosa pudica* Linn. and stem bark of *Symplocos racemosa* Roxb.

Parameters	***Mimosa pudica***	***Symplocos racemosa***
Stationary Phase	Merck Silica gel 60 F_{254} HPTLC precoated plates	
Mode of separation	Normal phase	
Mobile phase	Toluene:Ethyl acetate: Methanol: glacial acetic acid (8:1:0.5:0.3), (v/v/v/v)	Toluene: Methanol (8:1), (v/v)
Development chamber	Camag twin trough chamber	
Chamber saturation	20 minutes	
Sample applicator	Camag Linomat IV	
Band width	7mm	6mm
Space between the bands	10mm	10mm
Rate of sample application	15 secs/µL	15 secs/µL

Development distance	85 mm	85 mm
Derivatisation	10 % Methanolic Sulphuric acid reagent	
Densitometric scanner	Camag Scanner II WinCats 3 Software	
Lamp, Wavelength	Mercury, 366 nm	

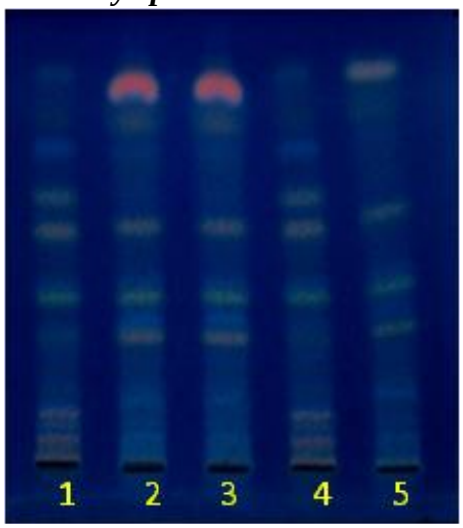
Figure 10: Chromatographic fingerprint of *Mimosa pudica* Linn. and *Symplocos racemosa* Roxb.

Mimosa pudica



Track 1- (Rajapur)
Track 2- (Nagothane)
Track 3- (Hativale)
Track 4- (Alibaug)
Track 5- (Kolhapur)
Track 6- (Yeoor)
Track 7- (Mangalore)

Symplocos racemosa



Track 1- (Mahabaleshwar)
Track 2- (Bhopal)
Track 3- (Madhya Pradesh)
Track 4- (Mumbai)
Track 5- (Varanasi)

Table 11: Optimized chromatographic HPLC fingerprint conditions for whole plant of *Mimosa pudica* Linn. and stem bark of *Symplocos racemosa* Roxb.

Specification	***Mimosa pudica***	***Symplocos racemosa***
Pump	Jasco HPLC-PU 980 pump	
Detector	Jasco, MD 910 multi-wavelength (PDA) detector.	
Column	Cosmosil C_{18}, 150 mm × 4.6 mm i.d., 5µ	
Injection volume	20 µl	
Wavelength	Max absorbance for plant fingerprint	
Mobile phase	Water: Acetonitrile (95:5 v/v) pH 3.0	Acetonitrile: distilled water (85: 15, v/v)

Figure 11. HPLC fingerprint of *Mimosa pudica* whole plant

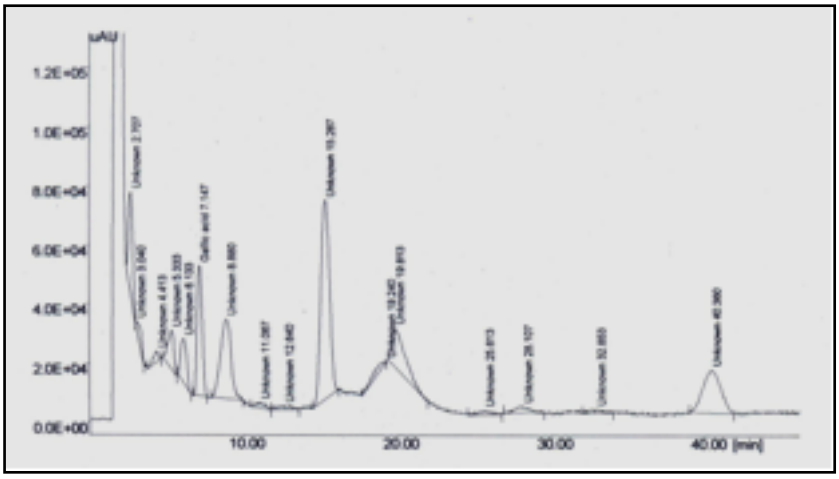
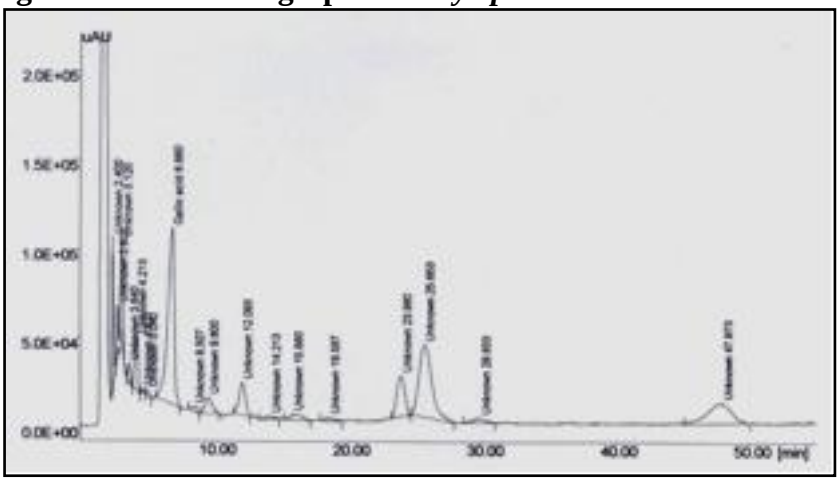


Figure 12. HPLC fingerprint of *Symplocos racemosa* stem bark



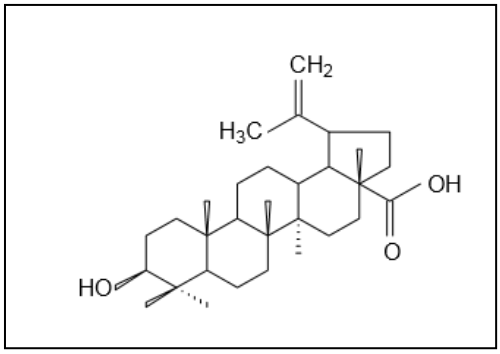
OBSERVATION: - Common reverse phase HPLC method was developed for establishing fingerprint pattern for Mimosa pudica whole plant and Symplocos racemosa stem bark in which Mimosa pudica gave a good separation of 16 bands where as Symplocos racemosa showed 18 bands respectively.

12. Quantitation and validation of Betulinic acid from *Symplocos racemosa* and Gallic acid from *Mimosa pudica* using HPTLC and HPLC techniques

A, Identification of Betulinic acid as a marker from *Symplocos racemosa*

Betulinic acid, pentacyclic triterpenoid, is the bioactive phytoconstituent from bark of *S. racemosa*. Betulinic acid has a wide range of biological and medicinal properties, including anti-human immunodeficiency virus (HIV), antibacterial, antimalarial, anti-inflammatory, anthelmintic, antinociceptive, anti-herpes simplex viruses-1 (HSV-1), immune-modulatory, antiangiogenic, and anticancer activity (Sook et al, 2015). The objective of this study was firstly to develop an HPTLC and HPLC technique, which allowed rapid, simple identification and quantification of Betulinic acid in the plant material in order to demonstrate their quality.

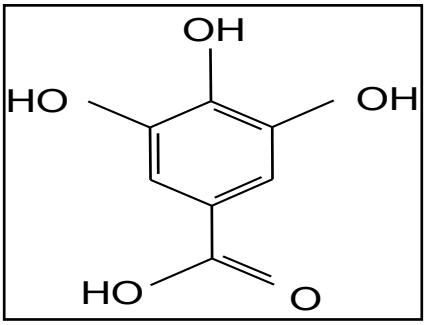
Figure 13. Structure of Betulinic acid



B. Identification of Gallic acid as a marker from *Mimosa pudica*

Gallic acid is a tri hydroxy benzoic acid, a type of phenolic acid. It is also known as 3, 4, 5 tri hydroxyl benzoic acid. Gallic acid is a naturally abundant phenolic compound in vegetables, such as asparagus, broccoli and aubergine. Gallic acid is known to have anti-inflammatory, antimutagenic, anticancer and antioxidant activity. It also seems to have antifungal, antiviral and antibacterial properties. Gallic acid has been found to show cytotoxicity against cancer cells without harming healthy cells. Gallic acid is used as a remote astringent in cases of internal haemorrhage. It has been found very beneficial in uterine, pulmonary, and nephritic haemorrhages. It has given benefit in purpura. It is used to treat albuminuria and diabetes. It is a known matrix-metalloproteinase inhibitor. All these properties make Gallic acid a pharmacologically important compound (Borde *et al*, 2011). The objective of this study was firstly to develop an HPTLC and HPLC technique, which allowed rapid, simple identification and quantification of Gallic acid in the plant material in order to demonstrate their quality.

Figure 14. Structure of Gallic acid



13. MATERIALS:

13.1 Plant material

The bark of *S. racemosa* was collected from Mahabaleshwar, Maharashtra and authenticated by Agharkar Research Institute, Pune (Authentication No. ARI 10-173) and a voucher specimen was deposited for further reference. Bark of *S. racemosa* was collected from different geographical regions of India to study regional variation in the content of betulinic acid. The whole plant of *M. pudica* was collected from Ratnagiri, Maharashtra and authenticated by Agharkar Research Institute, Pune (Authentication No. ARI 10-75) and a voucher specimen was deposited for further reference. Whole plant of *M. pudica* was collected from different geographical regions of India, to study regional variation in the content of gallic acid. Samples were shade dried for 7 days, then dried at 37±2ºC for 2 days, powdered in a mixer grinder, sieved through 85 mesh (BSS) and stored in air-tight containers.

13.2 Reference standards and chemicals:-

Betulinic acid (Purity ≥ 98%) was purchased from Sigma-Aldrich. Gallic acid (Purity ≥ 98%) was purchased from Sigma-Aldrich. HPLC grade solvents like methanol, acetonitrile, toluene, ethyl acetate, formic acid and distilled water were procured from Merck Specialties Pvt. Ltd., Mumbai, India.

13.3 Marketed sample:

The traditional Ayurvedic formulation, *Pushyanug churna* containing *S. racemosa* and *M. pudica* as one of the key ingredient (Baidyanath, Batch No. 150012), was purchased from Pharmacy in Mumbai.

14. METHODS:

14.1 Preparation of standard solution:

10.0 mg of standard (Gallic acid, Betulinic acid) was accurately weighed and transferred to 10.0 mL standard volumetric flask. The content was initially dissolved in minimum quantity of methanol, sonicated and then diluted up to the mark with methanol. The stock solution of 1000.0µg/mL was used to prepare working solutions of 100.0µg/mL, 10.0µg/mL and 1.0 µg/mL.

14.2 Optimization of Extraction Conditions for HPTLC and HPLC:

14.2.1 *Extraction condition for S. racemosa:*

Powder (1.0 g) of *S. racemosa* was extracted in ethyl acetate (5.0 mL) for HPTLC and methanol (10.0 mL) for HPLC into stoppered conical flask. The mixture was vortexed for 1 min, sonicated for 5 min, filtered through Whatman filter paper no.1 and then filtered through millipore filter (0.45 µm). The filtrates were used for further HPTLC and HPLC analysis.

14.2.2 ***Extraction condition for M. pudica****:*
Powder (1.0 g) of *S. racemosa* was extracted in ethyl acetate (5.0 mL) for HPTLC and methanol (10.0 mL) for HPLC into stoppered conical flask. Powder (1.0 g) of *M. pudica* was extracted in methanol (5.0 mL) for HPTLC and methanol (10.0 mL) for HPLC into stoppered conical flask. The mixture was vortexed for 1 min, sonicated for 5 min, filtered through Whatman filter paper no.1. The filtrates were used for further HPTLC and HPLC analysis.

14.2.3 ***Extraction conditions for marketed formulation (Pushyanug churna):***
The marketed formulation *Pushyanug churna* was extracted in ethyl acetate (5.0 mL) for HPTLC and methanol (10.0 mL) for HPLC in the ratio 1:10 (w/v) for Betulinic acid. The marketed formulation *Pushyanug churna* was extracted in methanol (5.0 mL) for HPTLC and methanol (10.0 mL) for HPLC in the ratio 1:10 (w/v) for Gallic acid. The mixture was vortexed for 1 min, kept standing overnight, sonicated for 5 min, filtered through Whattman filter paper no.1 and the filtrate was used for further HPTLC and HPLC analysis.

15. Validation of the method for quantitation of betulinic acid from *Symplocos racemosa* and gallic acid from *Mimosa pudica*:
The analytical method was validated for linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), specificity, and robustness, in accordance with ICH guidelines. Further statistical evaluations were performed.

15.1 ***Selectivity and specificity:***
Specificity of the method was ascertained by comparing the colour and R_f value of the band from betulinic acid and gallic acid with the relative band in the plant samples and marketed formulation.

15.2 ***System suitability****:*
System suitability experiment was performed by analyzing 20.0 µg/mL of betulinic acid and gallic acid and on a TLC plate (n=5) during the start of the method validation procedure. Values with % CV of ≤ 2 % were accepted.

15.3 ***Linearity*****:** Linearity of the method for estimation of betulinic acid and gallic acid was determined by analysing seven calibrant samples in triplicate on HPTLC plate. The mean response (area) values were plotted versus respective concentrations and a straight-line fit was made through the data points by least square regression analysis in order to obtain a linear regression equation.

15.4 ***Sensitivity****:*
Sensitivity of the method was determined in terms of LOD and LOQ values. Stock solution of betulinic acid and gallic acid was serially diluted with methanol to prepare the series of samples with least concentration and applied along with methanol as blank on TLC plate. The LOD and LOQ for betulinic acid and gallic acid were determined by measuring the signal to noise ratio (S/N). LOD and LOQ were considered at S/N of 3:1 and 10:1 respectively.

15.5 ***Precision****:*

Intra-day precision was evaluated by analyzing quality control samples in triplicate on the same day while inter-day precision was assessed by analyzing quality control samples in triplicate on three consecutive days. The % CV was taken as a measure of precision. Accuracy values within the range of 85 % to 115 % and % CV of ≤ 2 % were accepted.

15.6 *Stability:*
Stock solution of gallic acid (20.0 µg/mL) stored at 4 ± 1°C was tested for short term (12.00 hours) and for long term (15 days) stability. Samples were analyzed in triplicate and the values obtained were compared with the freshly prepared gallic acid of same concentration. Similarly, stock solution of gallic acid (20.0 µg/mL) stored at room temperature was subjected to bench top stability testing for 6.00 hours. Values within a difference range of ± 5 % were accepted.

15.7 *Ruggedness:*
Ruggedness of the method was assessed by incorporating deliberate small variations in the optimized chromatographic conditions. Effect of change in analyst, change in mobile phase composition on the response and R_f value of quality control samples was observed during the analysis and the results were expressed in terms of % mean difference. Values within a difference range of ± 5 % were accepted.

15.8 *Recovery:*
Accuracy/recovery of the method was evaluated using standard addition method. Quality control samples of betulinic acid and gallic acid were spiked into the powders of the given samples. Finally, four different extracts namely blank (unspiked), LQC-spiked, MQC-spiked and HQC-spiked were obtained. The % recovery of the quality control samples was calculated using the following formula:

$$\% \text{ Recovery} = \frac{\text{Amount found}}{\text{Original amount}} \times 100$$

Values within the range of 90 % to 110 % were accepted.

16. Assay and Method Application
The developed method was used to determine the content of betulinic acid from samples of *S. racemosa* and gallic acid from *M. pudica* collected from different regions of India and Maharashta and from the marketed Ayurvedic formulation *Pushyanug churna*. Relative retention factor and relative peak area of each characteristic peak from the samples of *S. racemosa and M. pudica* and its formulation related to the peak from betulinic acid and gallic acid were calculated using regression equation. The quantitative expression of the analyte in the chromatographic pattern of *S. racemosa* and *M. pudica* was derived. Microsoft Excel was used to determine mean, standard deviation, relative standard deviation and mean difference during the analysis.

17. Results and Observations:

Table12. Optimized chromatographic HPTLC conditions for quantitation of betulinic acid from stem bark of *Symplocos racemosa* Roxb and gallic acid from *Mimosa pudica*

Parameters	***Symplocos racemosa***	***Mimosa pudica***
Stationary Phase	Merck Silica gel 60 F_{254} HPTLC precoated plates	
Mode of separation	Normal phase	
Mobile phase	Toluene: Acetone: Ethyl acetate, 9:1:0.3, (v/v/v)	Toluene: Ethyl acetate: Formic acid, 2:7:1, (v/v/v)
Development chamber	Camag twin trough chamber	
Chamber saturation	20 minutes	
Sample applicator	Camag Linomat IV	
Band width	7 mm	
Space between the bands	7 mm	
Rate of sample application	15 secs/µL	
Development distance	85 mm	
Derivatisation	1% Anisaldehyde reagent	No derivatising reagent
Densitometric scanner	Camag Scanner II WinCats 3 Software	
Lamp, wavelength	Tungsten, 550nm	Deuterium, 278nm

Figure 15. Representative HPTLC plate photo and chromatogram showing gallic acid

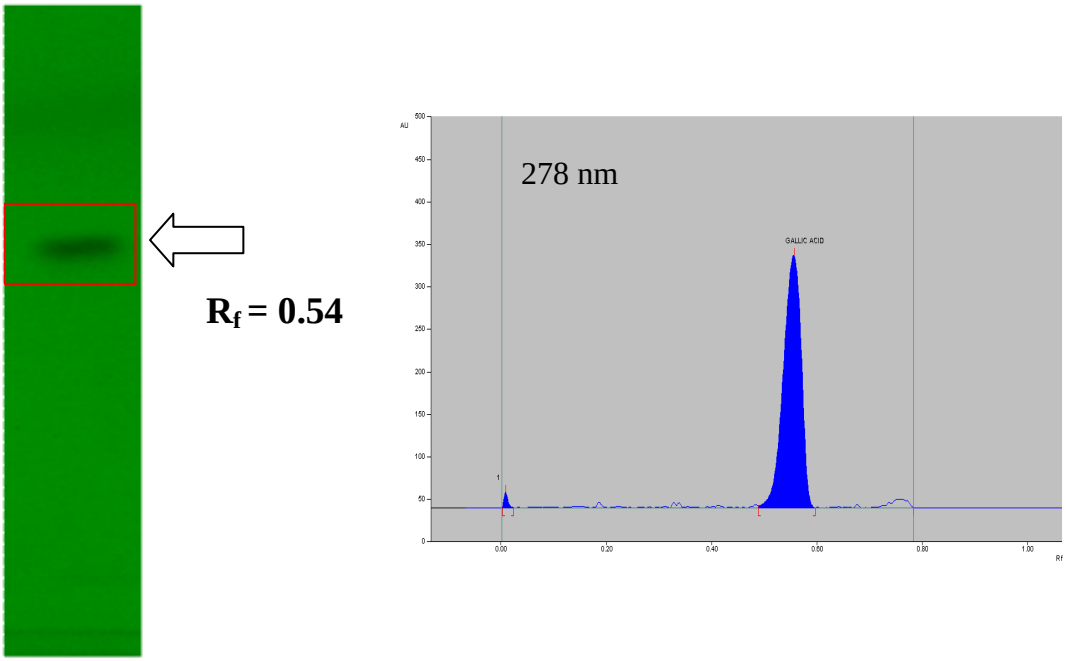
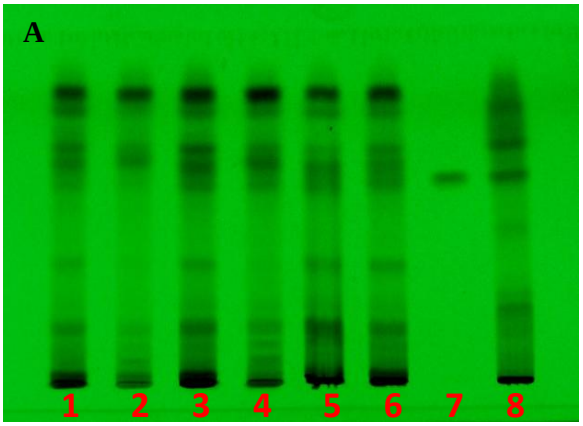
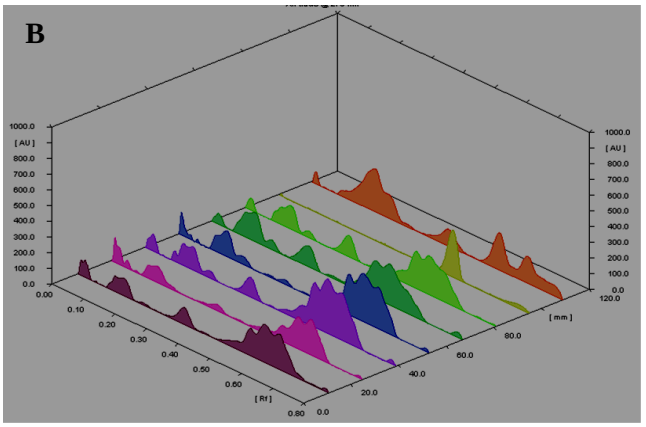


Figure 16. Representative HPTLC plate photo (A) and 3D overlay (B) showing gallic acid from *Mimosa pudica* collected from different regions and traditional marketed formulation (*pushyanug churna*) at 278 nm.





Track 1- Rajapur
Track 2- Nagothane
Track 3- Hativale
Track 4- Kolhapur
Track 5- Yeoor
Track 6- Mangalore
Track 7- Gallic acid (100ppm)
Track 8- Pushyanug churna

Figure 17. Representative HPTLC chromatogram showing gallic acid from *Mimosa pudica* at 278nm.

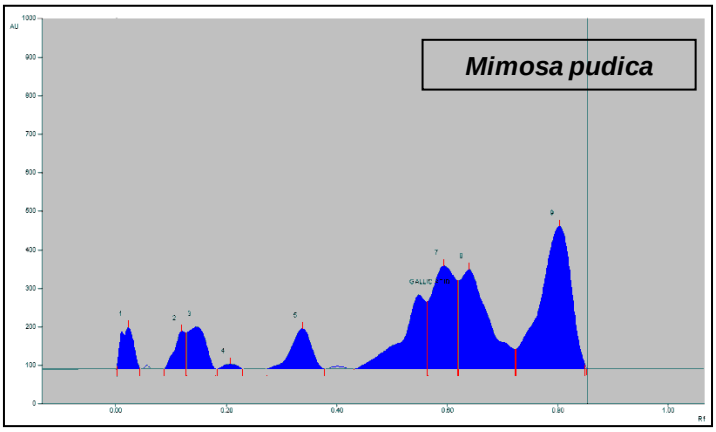


Table 13: Result of assay and method application for gallic acid using HPTLC

Sample	Content of gallic acid (mg/g)
Rajapur	0.4172 ± 0.0088
Nagothane	0.2281 ± 0.0175
Hatevali	0.7376 ± 0.0466
Kolhapur	0.5640 ± 0.0388
Yeoor	0.9009 ± 0.0474
Mangalore	0.6107 ± 0.0123
Pushyanug churna	0.5046 ± 0.254

Figure 18. Representative HPTLC plate photo and chromatogram showing betulinic acid

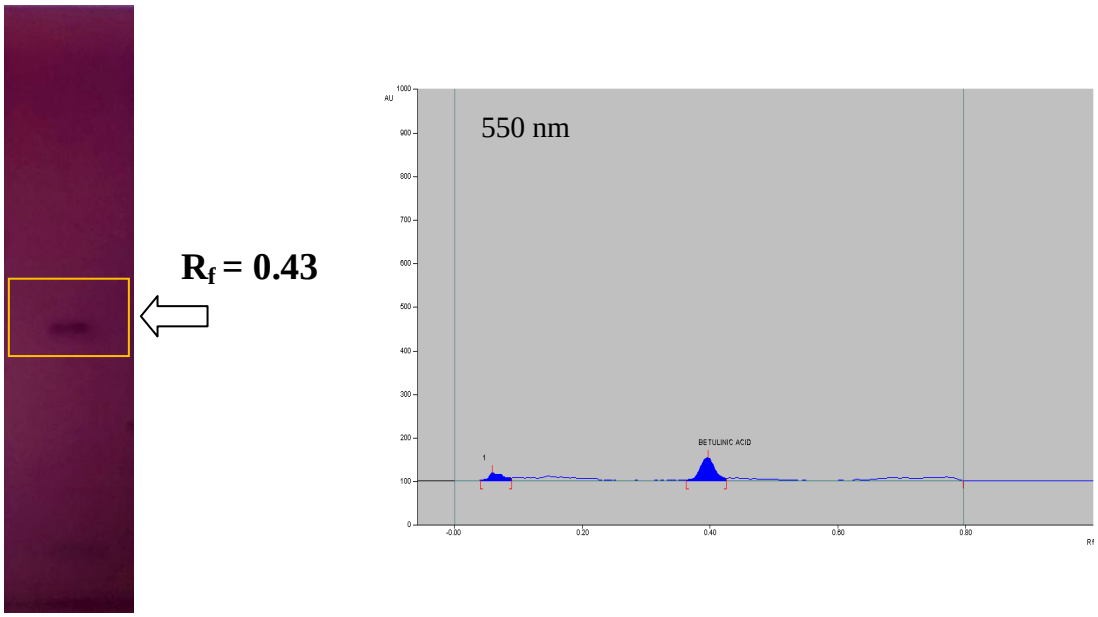
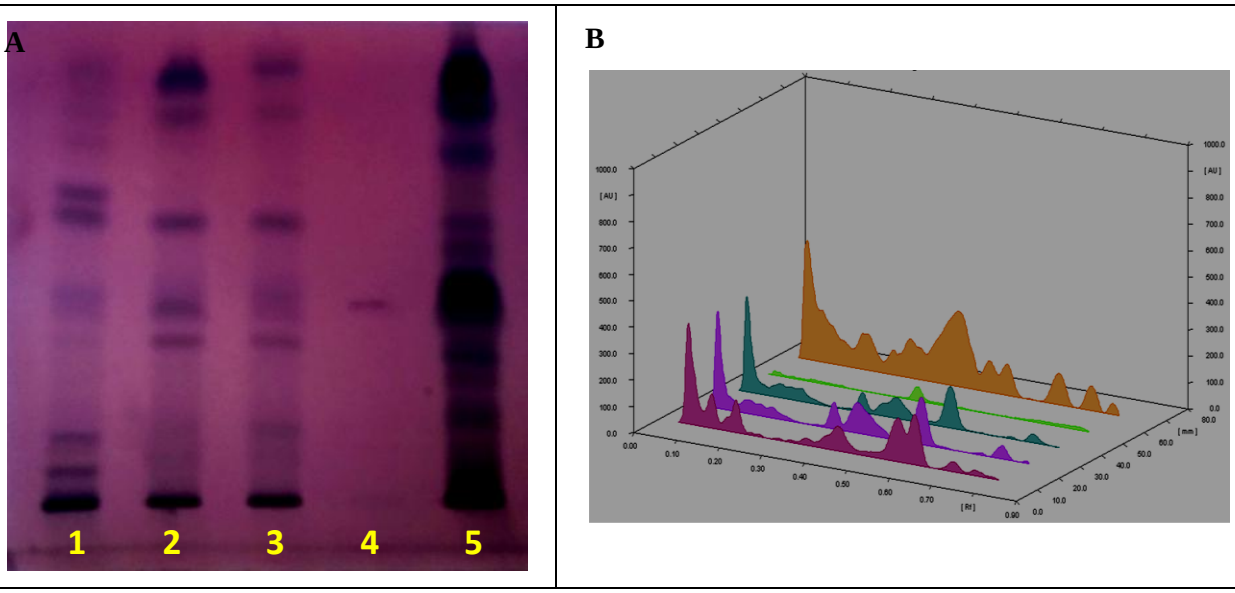


Figure 19. Representative HPTLC plate photo (A) and 3D overlay (B) showing betulinic acid from *Symplocos racemosa* collected from different regions and traditional marketed formulation (*pushyanug churna*) at 278 nm.



Track 1- Mahabaleshwar Track 2- Madhya Pradesh Track 3- Varanasi	Track 4- Betulinic acid (10ppm) Track 5- Pushyanug churna (Baidyanath, Mumbai)

Figure 20. Representative HPTLC plate photo showing betulinic acid from *Symplocos racemosa* at 550nm.

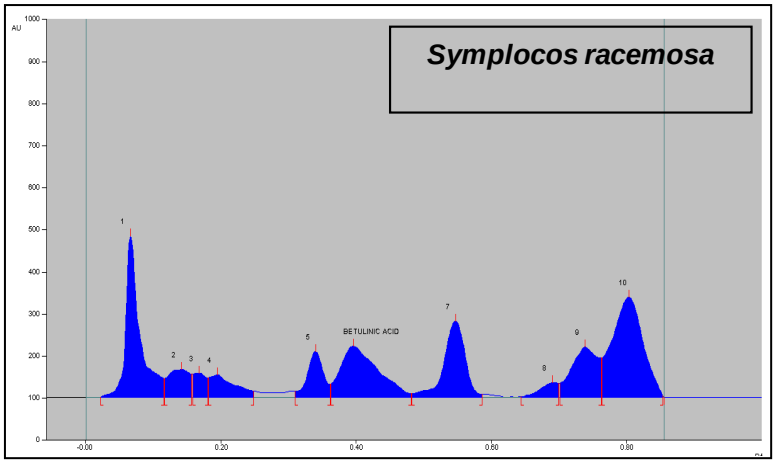


Table 14: Result of assay and method application for betulinic acid using HPTLC

Sample	Content of betulinic acid (mg/g)*
Mahabaleshwar	0.1668 ± 0.0364
Madhya Pradesh	0.2516 ± 0.0370
Varanasi	0.1708 ± 0.0175
Pushyanug Churna (PC)	0.9662 ± 0.0347

Table 15. Optimized chromatographic HPLC conditions for quantitation of betulinic acid from stem bark of *Symplocos racemosa* Roxb and gallic acid from whole plant of *Mimosa pudica*.

Specification	*Symplocos racemosa*	*Mimosa pudica*
Pump	Jasco HPLC-PU 980 pump	
Detector	Jasco, MD 910 multi-wavelength (PDA) detector	
Column	Cosmosil C_{18}, 150 mm × 4.6 mm i.d., 5µ	
Injection volume	20 µL	
Wavelength	210 nm	215 nm
Mobile phase	Acetonitrile: distilled water (85: 15, v/v)	Distilled water : Acetonitrile (95:5, v/v) with pH 3.0

Figure 21. Representative HPLC chromatogram showing gallic acid from Mimos*a pudica* at 215 nm.

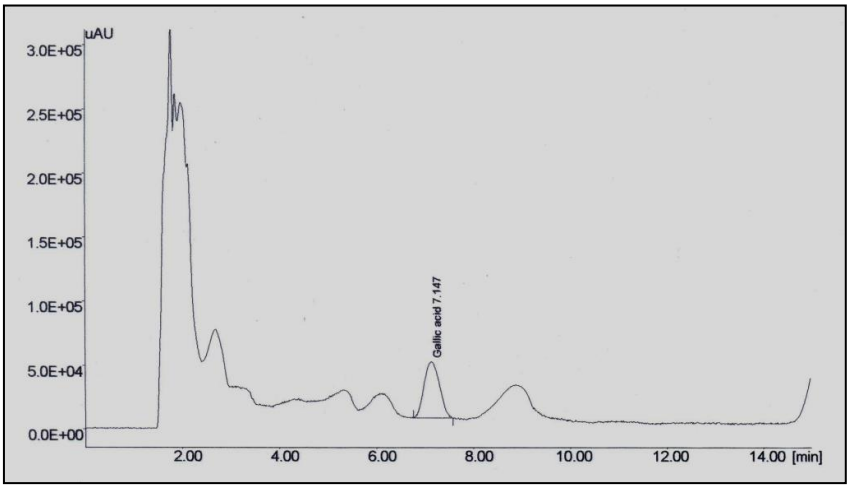


Figure 22. Representative HPLC chromatogram showing betulinic acid from *Symplocos racemosa* at 210 nm.

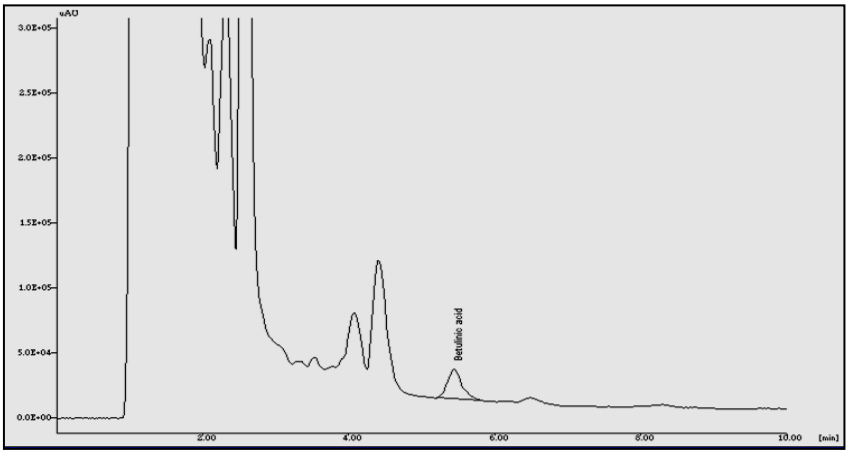


Figure 23. Calibration curve of betulinic acid and gallic acid using HPTLC and HPLC techniques

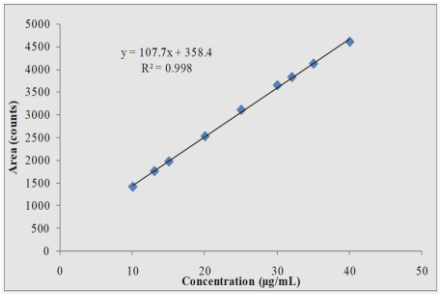
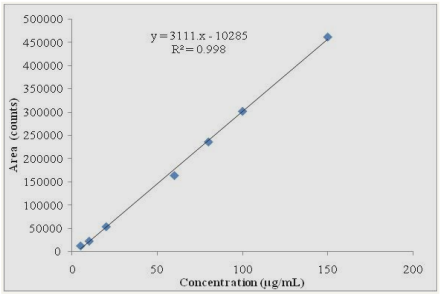
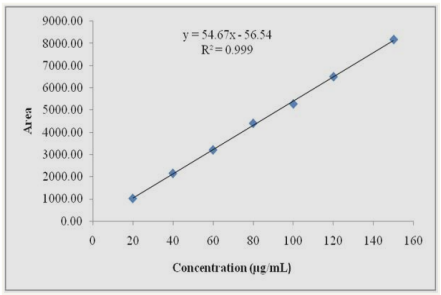
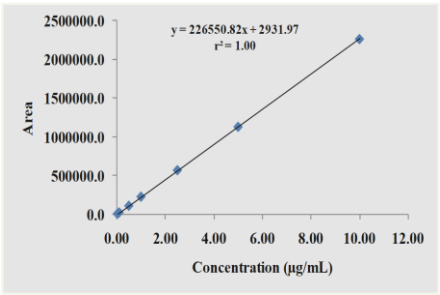
HPTLC	HPLC
Betulinic acid	Betulinic acid
	
Gallic acid	Betulinic acid
	

Table 16: Results of validation of betulinic acid and gallic acid using HPTLC technique

Parameters	Betulinic acid	Gallic acid
R_f	0.43	0.56
LOD and LOQ (µg/mL)	5 and 10	10 and 20
Linear Range (µg/mL)	10-40	20-150
Regression equation	y=107.7.x + 358.4	y= 54.67.x-56.54
Correlation co-efficient (r^2)	0.998	0.999
System Suitability (%CV)	1.55	1.97
Intra and interday precision (%CV)	1.3001 and 0.87	0.98 and 1.4033
Recovery (%)	80.18	82.56
Specificity	Specific	Specific
Ruggedness	Rugged	Rugged

Table 17: Results of validation of betulinic acid and gallic acid using RP-HPLC technique

Parameters (n=3)	Betulinic acid		Gallic acid	
Linearity (µg/mL)	5.0 – 150		0.03 - 10.0	
Regression equation	y=3111.x -10285		y = 226550.82x + 2931.97	
Correlation coefficient (r^2)	0.998		0.999	
Sensitivity				
LOD (µg/mL) LOQ (µg/mL)	2.5 5.0		0.01 0.03	
System suitability (% RSD , n=5)				
Rt (min)	0.80		1.54	
Area	1.44		0.25	
Precision (% RSD, n=5)				
Parameters	Area	R_t	Area	R_t
Intra-day	0.68 - 1.53	0.42 - 1.05	0.09 - 1.84	0.11 – 1.38
Inter-day	0.61 - 1.57	0.80 - 0.99	0.02 - 1.23	0.08 – 1.17

Table 18: Results of robustness for betulinic acid by variation in analyst, batch of column, flow rate and mobile phase composition

Parameter (n=3)	Change	R_t		Area	
		% RSD	% Mean difference	% RSD	% Mean difference
Analyst	Analyst1	0.14-0.89	--	0.79-1.94	--
	Analyst 2	0.11-1.75	0.30 - 3.98	0.64-1.50	0.11 - 1.54
Column	Column 1	1.00-1.49	--	0.47-1.62	--
	Column 2	0.36-1.27	-3.86 - 1.79	0.78-1.08	-3.11 - 2.49
Flow rate	0.9 ml/min	1.26-3.91	-1.67 -(-0.04)	0.15-1.47	-1.88 - 1.78
	1.0 ml/min	0.38-1.60	--	1.40-1.73	--
	1.1 ml/min	0.10-1.29	-0.41 - 0.61	1.06-1.93	-4.45 - 1.45
Mobile phase	I: 84:16 (v/v)	0.00-1.42	0.25 - 3.21	1.54-1.86	-3.22 - (-0.85)
	II: 85:15 (v/v)	0.45-0.67	--	0.76-1.58	--
	III: 86:14 (v/v)	0.39-0.78	-2.22 -(-0.16)	0.80-1.68	-2.69 - (-1.15)

Mobile phase I: (ACN: D/W), 84:16 (v/v)
Mobile phase II: (ACN: D/W), 85:15 (v/v)
Mobile phase III: (ACN: D/W), 86:14 (v/v)

Table 19: Results of robustness for gallic acid by variation in analyst, batch of column, flow rate and mobile phase composition

Parameter (n=5)	Change	R_t		Area	
		% RSD	% Mean difference	% RSD	% Mean difference
Analyst	Analyst1	1.16-1.61	--	0.38-1.73	--
	Analyst 2	1.18-1.84	-0.43- 0.85	0.27-1.85	-1.23-0.43
Column	Column 1	0.31-1.30	--	0.08-1.47	--
	Column 2	0.11-0.68	-0.29-0.19	0.16-1.88	-1.43-0.34
Flow rate	0.9 ml/min	0.45-0.70	-2.75-(-2.27)	0.56-1.69	0.05-1.03
	1.0 ml/min	0.41-0.66	--	0.88-1.58	--
	1.1 ml/min	0.46-0.60	0.86-1.70	0.94-1.68	-1.26-0.70
Mobile phase	I	0.35-0.53	-2.71-(-2.12)	0.77-0.87	-0.93-(-0.81)
	II	0.31-0.49	--	0.92-1.27	--
	III	0.50-0.63	0.61-2.13	0.10-1.10	0.56-1.96

Mobile phase I: 10 mM KH_2PO_4 in water: acetonitrile + 0.05% orthophosphoric acid, 96: 4: 0.05, v/v/v
Mobile phase II: 10 mM KH_2PO_4 in water: acetonitrile + 0.05% orthophosphoric acid, 95: 5: 0.05, v/v/v
Mobile phase III: 10 mM KH_2PO_4 in water: acetonitrile + 0.05% orthophosphoric acid, 94: 6: 0.05, v/v/v

18. Method Application: Estimation of betulinic acid and gallic acid from *Pushyanug churna* (*Ayurvedic* formulation)

The method was further applied to an Ayurvedic formulation *Pushyanug churna* containing *S. racemosa* and *M. pudica* as one of the key ingredient. A single peak was observed at the same retention time and retention factor in the sample of *Pushyanug churna* (Figure 18). There was no interaction between marker compound and other excipients present in the formulation of *Pushyanug churna*. The content of betulinic acid and gallic acid in *Pushyanug churna* is listed in the table below.

Figure 24. Representative HPLC chromatogram showing gallic acid and betulinic acid from traditional marketed formulation (*Pushyanug churna*)

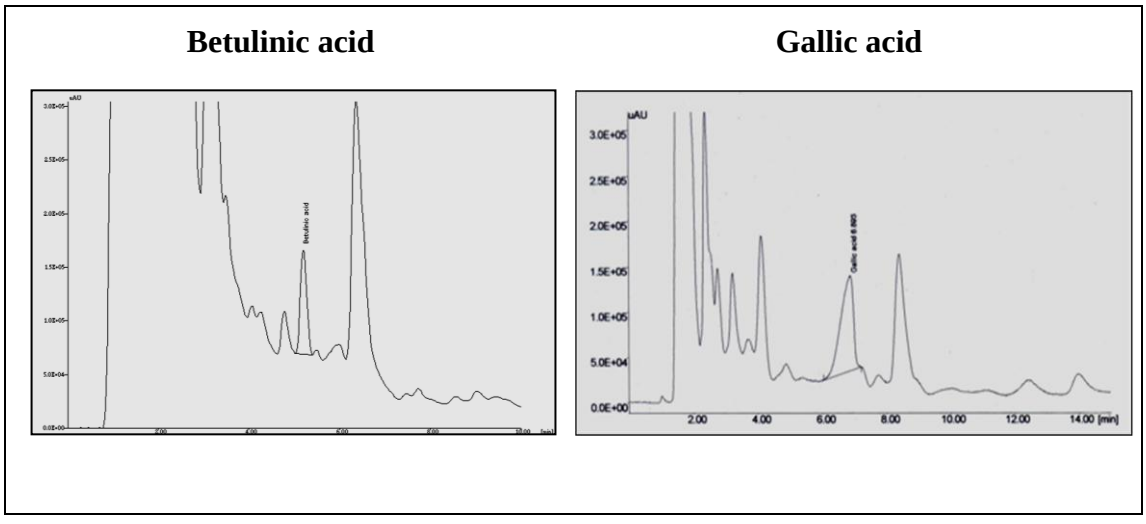


Table 20: Result of method application for betulinic acid and gallic acid using HPLC

Sample	Content of betulinic acid (mg/g)	Content of gallic acid (mg/g)
Pushyanug Churna (PC)	2.5486 ± 0.03933	0.828 ± 0.00756

19. DISCUSSION

Plant constituents vary considerably depending on several factors like temperature, light, drying, packing, storage etc. (Thakur et al., 2011). These variations impair not only the quality of phytotherapeutic agents but also their therapeutic value. The source and quality of raw materials play a pivotal role in ensuring the quality and stability of herbal formulations (Calixto, 2000). Thus, standardization and quality control of raw material and the final herbal formulations is of utmost importance (Gautam et al., 2010). In the development of the HPLC method for estimation and quantitation of betulinic acid and gallic acid, different chromatographic conditions were tried to improve the separation of marker (betulinic acid and gallic acid) from other peaks in suitable time. Betulinic acid was optimally detected and quantified by using RP-HPLC-PDA with acetonitrile and distilled water (85:15, v/v) and gallic acid was detected with Water :Acetonitrile (95:5) with pH 3.0. The retention time (R_t) of betulinic acid was found to be 5.2 ± 0.002 min and for gallic acid it was 6.9 ± 0.004 min, under optimized chromatographic conditions. The specificity of the intended method was established by comparing the retention time and absorption spectra of the target peaks from the analyzed samples with the reference compound. The limit of detection (LOD) and limit of quantification (LOQ) was found to be 2.5µg/mL and 5.0µg/mL for betulinic acid, and 0.01µg/mL and 0.03µg/mL, respectively suggesting adequate sensitivity of the method. The percent coefficient of variations during the system suitability study was found to be 0.80 and 1.44% respectively for retention time and response of betulinic acid; 1.54 and 0.25 for gallic acid. The response for the marker was found to be linear in the range of 5.0 - 150 µg/mL with a coefficient of determination of 0.998 for betulinic acid; 0.03 - 10 µg/mL with a coefficient of determination of 0.999 for gallic acid From the above results, it can be said that betulinic acid showed a broad range of linear detection. This resulted in a regression equation, y = 3111.8x - 10285 for betulinic acid and y = 226550.82x + 2931.97 for gallic acid. Inter and intra-day precision for the quality control samples of betulinic acid and gallic acid was within the acceptance limit of 85-115%. Mean recovery for the quality control samples of betulinic acid and gallic acid was found to be 85.92%

and 80.35%, respectively. Betulinic acid and gallic acid was found to be stable for 6 h at ambient temperature and for 15 days below 8°C. The method was found to be simple, rapid, specific, precise and rugged as the values obtained were within the acceptance limits. The developed and validated method was applied for the estimation and quantitation of betulinic acid and gallic acid from the extract of *S. racemosa and Minosa pudica* respectively and from an Ayurvedic formulation, *Pushyanug churna*.

20. Toxicity study

Toxicology has been defined as the study of the adverse effects of xenobiotics and thus is a borrowing science that has involve from ancient practitioners. Modern toxicology goes beyond the study of the adverse effects of exogenous agents to the study of molecular biology, using toxicants as tools. Currently many toxicologists are studying the mechanisms of endogenous compounds such as oxygen radicals and other reactive intermediates generated from xenobiotics and endobiotics. Historically toxicology formed the basis of therapeutic and experimental medicines. A toxic substance is defined by the National Institute for Occupational Safety and Health as "One which demonstrates the potential to induce cancer; produce long term disease or bodily injury; to affect health adversely; to produce acute discomfort; or to endanger the life of man or animals through exposure via the respiratory tract, skin, eyes, mouth or other routes.

Toxicity tests may be classified as follows:

- Acute toxicity test
- Sub-acute toxicity test
- Sub-chronic toxicity test
- Chronic toxicity test

As part of the efforts of quality assurance of herbal products, scientific experiments are to be carries out to assess the safety of the medicinal plants using the rodent as an alternative mammalian. Minimal toxicological studies and clinical trials are required with emphasis on testing of drugs in the manner they are used in traditional medicines with due importance given to their pharmaceutical forms.

In the present work acute toxicity study of ingredient plants viz. *Mimosa pudica* and *Symplocos racemosa* was conducted using Revised Draft Guideline 420, Acute Oral Toxicity Fixed Dose Procedure as per the recommendations of the OECD guidelines for testing of chemicals (OECD, 2000). The main objective of this study was to find out whether the ingredient plants show any toxic effects when administered individually and separately as test material.

Table 21. Protocol for Animal Toxicity Study

Test Material	Ethanolic extract of *Mimosa pudica a*nd *Symplocos racemosa*
Animal model	Albino Swiss mice
Animal procurement	Bharat Serums and Vaccines Pvt Ltd, Mumbai
Sex	Female
Weight of animals	Between 18-22g
No. of Dose groups	Two
Animals per group	Four
Route of administration	Oral administration using Gavage no. 16
Dose volume	2.0mL per animal
Vehicle for administration	Distilled water
No. of administration	Single
Dose	50mg/kg, 300mg/kg, 2000mg/kg
Study duration	Acclimatization for 14 days, before dosing and 14 days observation period after dosing.
Parameters to be observed	Cage side observations, daily food and Water intake, daily body weight and mortality record.
Study site	Animal Testing Unit, Ramnarain Ruia College, Mumbai (CPCSEA / 315)

20.1. Animal Maintenance

Animals are housed in groups of four animals for each dose in solid floor polypropylene cages with rice husk bedding. With the exception of an overnight fast, immediately before dosing and for approximately two hours after dosing, free access to drinking water and food was allowed throughout the study. The animal room was at ambient temperature of 30- 32˚C and relative humidity of 65-70%. The rate of air exchange is continuous through a system of inlet for fresh air and outlet for bad air. The lighting is controlled by a time switch to give 12 hrs continuous light and 12 hrs continuous dark cycles. The animals are provided with drinking water *ad libitum* and are fed on commercially available rat feed supplied by AMRUT FEED.

Table 22. Dose Regimen for Acute Toxicity Study

Group	Sex	Dose mg/kg Body weight	No. of animals used		Total Volume Administered per Animal (cm^3)
			Sighting Study	Main Study	
I	Female	Nil	-	04	2 cm^3 D/W
II a	Female	50	01	04	2 cm^3
II b	Female	50	01	04	2 cm^3
III a	Female	300	01	04	2 cm^3
III b	Female	300	01	04	2 cm^3
IV a	Female	2000	01	04	2 cm^3
IV b	Female	2000	01	04	2 cm^3

a- Ethanolic extract of *Mimosa pudica* Whole plant powder

b- Ethanolic extract of *Symplocos racemosa* Stem bark powder

A control group (Group I) was also maintained comprising of 4 females. The animals of control group were administered 2cm^3 of distilled water. Animals were subjected to fasting, food but not water was withheld 3-4 hours prior to dosing (OECD, 2000). All animals were dosed orally once only by gavage no. 16 using a metal cannula attached to a graduated syringe. The animals from Group II (a) were given ethanolic extract of 50 mg/kg (low dose) of whole plant powder of *Mimosa pudica* in 2cm^3 of distilled water. The animals from Group II (b) were given ethanolic extract of 50 mg/kg (low dose) of stem bark powder of *Symplocos racemosa* in 2cm^3 of distilled water. The animals from Group III (a) were given ethanolic extract of 300 mg/kg (intermediate dose) of whole plant powder of *Mimosa pudica* in 2cm^3 of distilled water. The animals from Group III (b) were given ethanolic extract of 300 mg/kg (intermediate dose) of stem bark powder of *Symplocos racemosa* in 2cm^3 of distilled water. The animals from Group IV (a) were given ethanolic extract of 2000 mg/kg (high dose) of whole plant powder of *Mimosa pudica* in 2cm^3 of distilled water. The animals from Group IV (b) were given ethanolic extract of 2000 mg/kg (high dose) of stem bark powder of *Symplocos racemosa* in 2cm^3 of distilled water. The volume administered to each animal is calculated according to its fasting body weight at the time of dosing. Individual body weights, food and water are recorded throughout the study period.

21. Observations:

Table 23. Summary of cage side observations

Obs No.	Parameters	Observations
1.	Condition of the fur	Normal
2.	Skin	Normal
3.	Subcutaneous swelling	Nil
4.	Abdominal distension	Nil
5.	Eye dullness	Nil
6.	Opacity of the eyes	Nil
7.	Ptosis	Nil
8.	Colour and consistency of faeces	Nil
9.	wetness of soiling of the perineum	Nil
10.	Condition of teeth	Nil
11.	Breathing abnormalities	Nil
12.	Gait	Nil

21.2. Body Weight Changes

Body weight is an important factor to monitor the health of an animal. Loss of body weight is frequently the first indicator of the onset of an adverse effect. A dose that causes 10 % or more reduction in the body weight is considered to be the dose that produces minimum toxic effect, irrespective of whether or not it is accompanied by any other changes. All the animals from treated groups did not show any significant decrease in body weight for all the 14 days as compared with 0 day value indicating no signs of toxicity. Animals from all the dose groups did not show change in body weight greater than 10 % of their initial body weights.

Figure 25. Daily Body weight record (Normal Control)

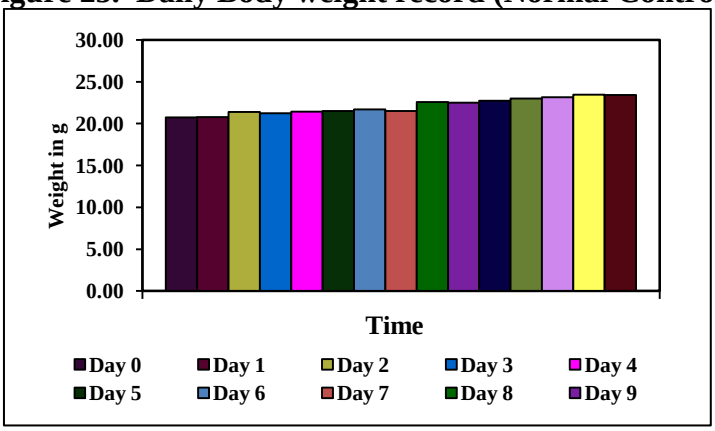


Figure 26. Daily Body weight record after *Mimosa pudica* treatment

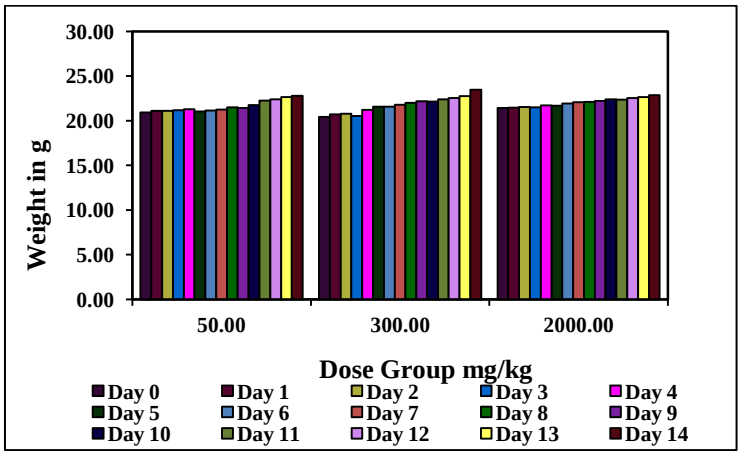
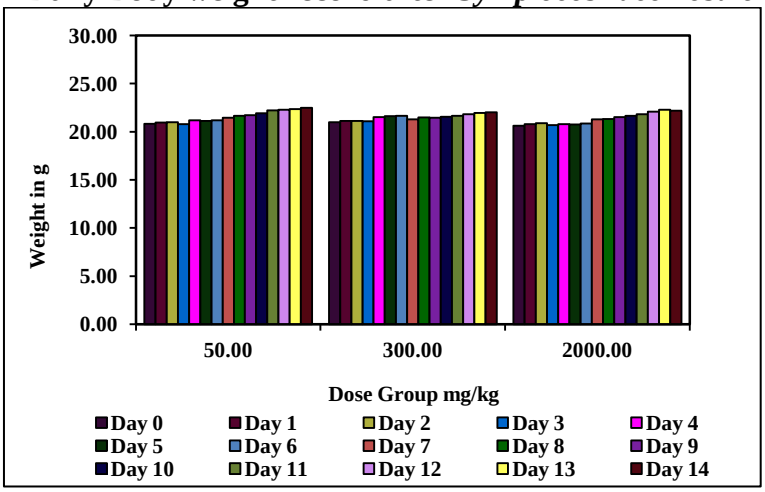


Figure 27 Daily Body weight record after *Symplocos racemosa* treatment



21.3. Food and Water Consumption

There are no significant changes in food and water intake of the test animals at all dose levels as compared to the control. The data for food consumption is given below.

Figure 28. Daily Water Consumption record (Normal Control)

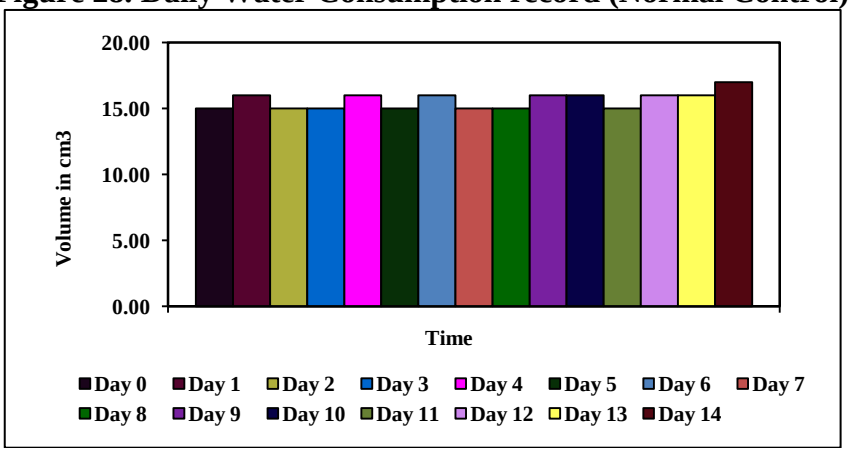


Figure 29. Daily Water Consumption record after *Mimosa pudica* treatment

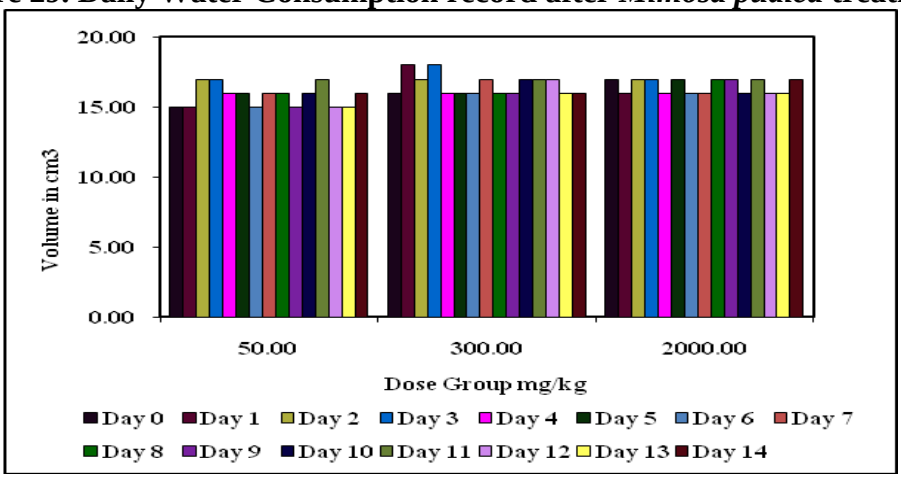
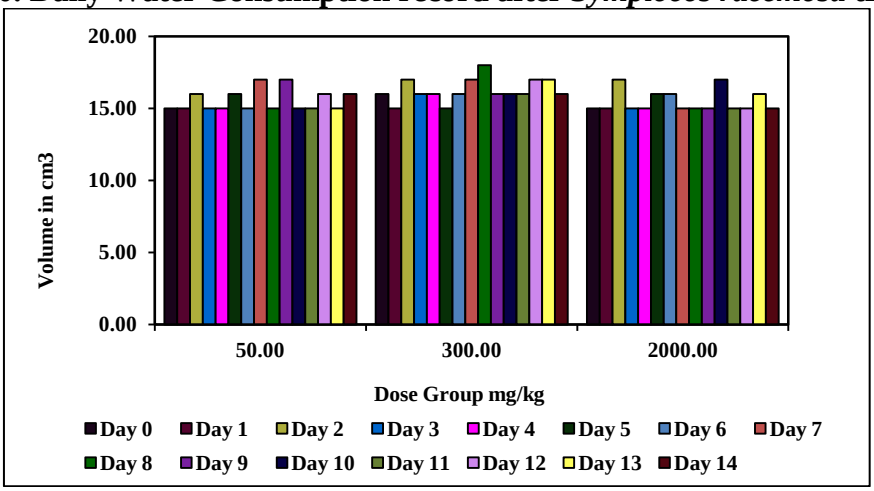


Figure 30. Daily Water Consumption record after *Symplocos racemosa* treatment



Food consumption can indicate an adverse effect of a drug at an early stage. Water consumption is significantly changed in studies of diuretic compounds that are known or expected to affect the kidneys. There are no significant changes in food and water consumption of the animals from all the dose groups.

21.4. Mortality

Mortality is the main criteria in assessing the acute toxicity (LD_{50}) of any drug. The highest dose as recommended by OECD guidelines, 2001 is used for the study. There is no mortality recorded even at the highest dose level; 2.0 g/kg body weight.

Table 24. Daily Body weight record of Normal Control in grams (Mean± S.D., n=5)

Sex	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Female	20.73 ± 0.61	20.78 ± 0.61	21.36 ± 0.15	21.25 ± 0.13	21.43 ± 0.17	21.53 ± 0.45	21.70 ± 0.44	21.53 ± 0.17	22.58 ± 0.73	22.49 ± 0.37	22.73 ± 0.52	23.01 ± 0.39	23.17 ± 0.46	23.46 ± 0.44	23.42 ± 0.38

Table 25. Daily Body weight record of treated animals in grams *Mimosa pudica* (Mean± S.D., n=5)

Dose groups mg/kg	Sex	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
50	Female	20.94 ± 0.41	21.09 ± 0.45	21.12 ± 0.39	21.16 ± 0.50	21.28 ± 0.45	21.02 ± 0.54	21.14 ± 0.54	21.26 ± 0.54	21.50 ± 0.51	21.44 ± 0.42	21.74 ± 0.50	22.26 ± 0.46	22.38 ± 0.50	22.63 ± 0.48	22.79 ± 0.48
300	Female	20.43 ± 0.40	20.71 ± 0.32	20.76 ± 0.36	20.54 ± 0.36	21.20 ± 0.43	21.58 ± 0.46	21.56 ± 0.46	21.78 ± 0.39	21.99 ± 0.35	22.17 ± 0.42	22.15 ± 0.46	22.39 ± 0.47	22.53 ± 0.47	22.76 ± 0.33	23.46 ± 0.25
2000	Female	21.41 ± 0.45	21.46 ± 0.47	21.52 ± 0.44	21.48 ± 0.41	21.72 ± 0.33	21.68 ± 0.36	21.92 ± 0.34	22.08 ± 0.46	22.10 ± 0.45	22.20 ± 0.45	22.38 ± 0.47	22.34 ± 0.48	22.55 ± 0.42	22.63 ± 0.50	22.88 ± 0.50

Table 26. Daily Body weight record of treated animals in grams *Symplocos racemosa* (Mean± S.D., n=5)

Dose groups mg/kg	Sex	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
50	Female	20.81 ± 0.55	20.97 ± 0.55	21.00 ± 0.47	20.80 ± 0.47	21.18 ± 0.52	21.13 ± 0.70	21.20 ± 0.63	21.47 ± 0.72	21.64 ± 0.64	21.70 ± 0.65	21.93 ± 0.59	22.22 ± 0.49	22.29 ± 0.41	22.36 ± 0.26	22.48 ± 0.23
300	Female	21.00 ± 0.46	21.12 ± 0.36	21.12 ± 0.46	21.10 ± 0.64	21.51 ± 0.40	21.61 ± 0.29	21.67 ± 0.30	21.28 ± 0.75	21.50 ± 0.30	21.46 ± 0.27	21.56 ± 0.27	21.64 ± 0.34	21.81 ± 0.32	21.94 ± 0.25	22.03 ± 0.24
2000	Female	20.61 ± 0.52	20.78 ± 0.52	20.89 ± 0.54	20.68 ± 0.53	20.78 ± 0.53	20.74 ± 0.56	20.86 ± 0.55	21.29 ± 0.55	21.32 ± 0.47	21.52 ± 0.46	21.64 ± 0.43	21.81 ± 0.44	22.10 ± 0.48	22.28 ± 0.49	22.20 ± 0.47

Table 27. Daily Food Consumption record of Normal Control in grams (Note: Values for each cage)

Sex	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Female	10.00	12.00	11.00	11.00	12.00	12.00	12.00	12.00	11.00	12.00	11.00	13.00	13.00	12.00	11.00

Table 28. Daily Food Consumption record of treated animals in grams *Mimosa pudica* (Note: Values for each cage)

Dose groups mg/kg	Sex	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
50	Female	10.00	11.00	10.00	11.00	10.00	11.00	10.00	12.00	11.00	12.00	11.00	10.00	11.00	12.00	11.00
300	Female	11.00	11.00	12.00	11.00	11.00	12.00	11.00	12.00	11.00	12.00	11.00	11.00	12.00	11.00	11.00
2000	Female	11.00	10.00	10.00	11.00	12.00	11.00	11.00	10.00	11.00	12.00	11.00	12.00	11.00	11.00	12.00

Table 29. Daily Food Consumption record of treated animals in grams *Symplocos racemosa* (Note: Values for each cage)

Dose groups mg/kg	Sex	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
50	Female	11.00	12.00	11.00	12.00	10.00	12.00	11.00	11.00	12.00	11.00	11.00	12.00	11.00	12.00	11.00
300	Female	11.00	12.00	11.00	12.00	12.00	11.00	13.00	12.00	12.00	11.00	12.00	12.00	11.00	12.00	11.00
2000	Female	11.00	10.00	11.00	10.00	11.00	11.00	11.00	11.00	10.00	12.00	10.00	11.00	10.00	11.00	11.00

Table 30. Daily Water Consumption record of Normal Control in mL (Note: Values for each cage)

Sex	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Female	15.00	16.00	15.00	15.00	16.00	15.00	16.00	15.00	15.00	16.00	16.00	15.00	16.00	16.00	17.00

Table 31. Daily Water Consumption record of treated animals in mL *Mimosa pudica* (Note: Values for each cage)

Dose groups mg/kg	Sex	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
50	Female	15.00	15.00	17.00	17.00	16.00	16.00	15.00	16.00	16.00	15.00	16.00	17.00	15.00	15.00	16.00
300	Female	16.00	18.00	17.00	18.00	16.00	16.00	16.00	17.00	16.00	16.00	17.00	17.00	17.00	16.00	16.00
2000	Female	17.00	16.00	17.00	17.00	16.00	17.00	16.00	16.00	17.00	17.00	16.00	17.00	16.00	16.00	17.00

Table 32. Daily Water Consumption record of treated animals in mL *Symplocos racemosa* (Note: Values for each cage)

Dose groups mg/kg	Sex	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
50	Female	15.00	15.00	16.00	15.00	15.00	16.00	15.00	17.00	15.00	17.00	15.00	15.00	16.00	15.00	16.00
300	Female	16.00	15.00	17.00	16.00	16.00	15.00	16.00	17.00	18.00	16.00	16.00	16.00	17.00	17.00	16.00
2000	Female	15.00	15.00	17.00	15.00	15.00	16.00	16.00	15.00	15.00	15.00	17.00	15.00	15.00	16.00	15.00

Table 33. Mortality record of Normal Control (Note: Values for each cage)

Sex	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Female	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

Table 34. Mortality record of animals treated with *Mimosa pudica* (Note: Values for each cage)

Dose groups mg/kg	Sex	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
50	Female	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
300	Female	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
2000	Female	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

Table 35. Mortality record of animals treated with *Symplocos racemosa* (Note: Values for each cage)

Dose groups mg/kg	Sex	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
50	Female	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
300	Female	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
2000	Female	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

22. Efficacy studies

Women's health is a topic of concern in the medical field, as women are an important factor in the reproduction of healthy progeny. The environmental factors, fast changing lifestyles and various addictions (drug abuse) as well as excess use of drugs (like steroids) have endangered their health. More than 4.5 million women (ages 18 to 50) report at least one gynecological condition each year (Mishra, 2004). Nearly half of the total female population in India belonging to weaker sections suffers from gynecological disorders owing to reasons associated with poverty, illiteracy and social taboo. Existing hormonal therapy used for gynecological disorders is believed by the masses to render harmful side effects. The present hormonal therapy is "accused" to impair some physiological activity and thus mitigates another disorder. Therefore the currently available therapies, which advocate greater use of antibiotics, steroids or surgery may not be an ultimate answer for a women's ill health. This has resulted in the popularization of herbal drugs as alternative medicine. Herbal medicines have been recognized as a valuable and readily available resource for primary health care and WHO has endorsed their safe and effective use (Jadhav and Bhutani, 2005; WHO 1993).

22.1. Evaluation of efficacy of plant extracts in Letrozole treated rats

The objectives for the efficacy studies are;

- To evaluate the efficacy of the individual plant material under investigation in comparison with a modern drug clomiphene citrate for the management of PCOS.
- **Methodology:**

  The Wistar female rats weighing 180-220g will be obtained from Bharat serums and vaccines Ltd, Thane, Mumbai. All animals will be kept under 27±2ºC, 80±10% humidity and a 12h light/12h dark cycle. The animals will be provided free access to water and were fed standard rat diet. Polycystic ovary (PCO) will be induced in the female rats by giving an oral administration of letrozole once daily at the concentration of 1.0 mg/kg dissolved in 1% carboxymethylcellulose (2mL/kg body weight, vehicle). The treatment period is of 21 days ((Kafali, *et al*, 2004). After the treatment period of 21 days, blood samples will be drawn to determine testosterone, estrogen, progesterone and total cholesterol. The animals of each group will be then given different treatments for recovery with the individual plants for 15 days post Letrozole induction. After the study period, they will be sacrificed according to the study protocol approved through IAEC committee.

Table 36: General Protocol for Evaluation of Efficacy

Animal model	Albino Wistar Rats
Animal procured from	Haffkine Biopharmaceuticals Limited., Parel, Mumbai
Sex	Female
Weight of animals	Between 175-180 g.
No. of dose groups	Seven
Animals per group	Six
Dose of Letrozole	1.0 mg/kg body weight (Kafali et al, 2004)
Route of administration	p.o. (Oral administration)
Vehicle for administration	1% aqueous solution of Carboxymethylcellulose (CMC)
Volume of vehicle	2.0 mL/kg body weight (Kafali et al, 2004)
Test Sample	Whole plant powder of Mimosa pudica, Stem bark powder of Symploco racemosa and tablet containing Clomiphene citrate (1 mg/kg body weight) (IDR, 2006)
Route of test sample administration	Oral
Dose volume	2.0 ml per animal
Vehicle for oral dose	Distilled water
Parameters for evaluation	Testosterone, Estrogen, Progesterone and Total Cholesterol, Histopathological evaluation of ovary under light and electron microscope.
Animal maintenance	The animals are maintained as per the procedure described earlier in the chapter of toxicity studies.
Study site	Animal Testing Unit, Ramnarain Ruia College, Mumbai (CPCSEA / 315).

Table 37: The animals were divided into the following dosage groups:

Group I	Normal control	Received 2 ml distilled water orally
Group II	Letrozole control	Sacrificed on day 22
Group III	Letrozole + Natural Recovery	Received 2 ml distilled water orally (for 15 days post Letrozole induction)
Group IV	Letrozole + Clomiphene citrate (Mode Drug)	Received 1.0 mg/kg body weight dose of Clomiphene citrate. (For 15 days post Letrozole indu
Group V	Letrozole + *Mimosa pudica*	Received 500 mg/kg body weight dose of *Mimosa pudica* whole plant powder. (For 15 days post Letrozole induction)
Group VI	Letrozole + *Symplocos racemosa*	Received 500 mg/kg body weight dose of *Symplocos racemosa* stem bark powder. (For 15 days post Letrozole induction)

22.2 Results

The plasma testosterone levels (ng/dL), estrogen levels (pg/mL), progesterone levels (ng/mL) and cholesterol levels (mg/dL) were recorded after Letrozole induction

after 21 days (i.e. on Day 22 of study period) and after plant treatment for 15 days (i.e on Day 37 of study period) The ovarian weights and uterine weights were also recorded at the termination of the study.

22.2.1 Testosterone levels

The plasma testosterone level in Normal control group i.e. Group I was observed to be around 235.82 ± 14.98 ng/dL on Day 22 and is maintained consistently up to 245.33 ± 13.84 ng/dL on Day 37 indicating the stable condition. After the animals were treated with Letrozole for the induction of PCOS, the mean testosterone level in Letrozole control group i.e Group II significantly increased up to 795.20 ± 24.10 ng/dL (Table 38, Figure 31).

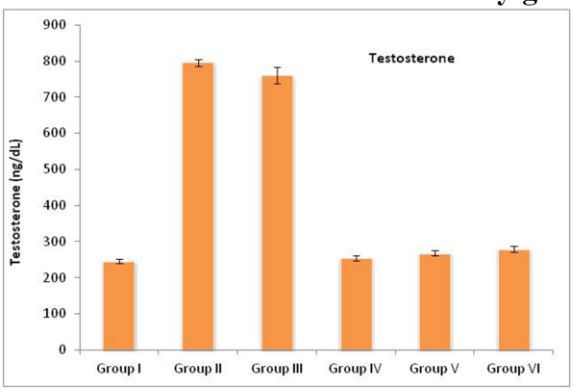
The mean plasma testosterone level of 781.10 ± 18.83 ng/dL was found after induction of PCOS with Letrozole for Natural recovery group i.e Group III. After 15 days post Letrozole induction, the testosterone level in the animals from natural recovery group was found to be 759.73 ± 55.39 ng/dL indicating no signs of recovery (Table 38, Figure 31).

In Clomiphene citrate treated group i.e Group IV, the testosterone level increased up to 716.37 ± 28.86 ng/dL when the animals were treated with Letrozole for the induction of PCOS. A decrease to 255.42 ± 17.43 ng/dL was observed on treatment with modern drug Clomiphene citrate for 15 days (Table 38, Figure 31).

The animals from *Mimosa pudica* treated group i.e. Group V showed increase in testosterone level up to 737.30 ± 26.57 ng/dL after the treatment with Letrozole and a decrease to 268.00 ± 18.58 ng/dL was observed after plant treatment (Table 38,Figure 31).

The induction of PCOS with Letrozole showed increase in testosterone level up to 755.23 ± 6.26 ng/dL in the plant treatment group of *Symplocos racemosa* i.e Group VI and the plant treatment for 15 days showed a reduction in testosterone level up to 279.95 ± 21.30 ng/dL (Table 38, Figure 31).

Figure 31: Testosterone levels (ng/dL) in the treated groups along with normal control, Letrozole Control and Natural recovery group



22.2.2 Estrogen levels

In normal control group i.e. Group I the estrogen level was found to be 56.17 ± 3.66 pg/mL on Day 22 and is maintained consistently up to 55.83 ± 5.19 pg/mL on Day 37 indicating the stable condition.

After Letrozole induction, the estrogen level significantly decreased to 29.88 ± 3.64 pg/mL in Letrozole control group i.e. Group II (Table 38, Figure 32).

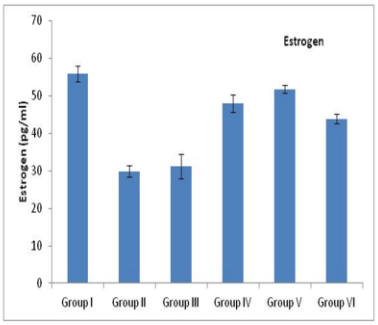
In natural recovery group i.e. Group III, a decrease in estrogen level to 34.67 ± 4.03 pg/mL was observed when the animals were treated with Letrozole and after 15 days post Letrozole induction the estrogen level was found to be 31.17 ± 7.94 pg/mL indicating no signs of recovery (Table 38, Figure 32).

The estrogen level decreased to 27.33 ± 3.56 pg/mL in Clomiphene citrate group i.e Group IV after Letrozole treatment and after the treatment with modern drug Clomiphene citrate for 15 days post Letrozole induction, the increase in estrogen level to 48.00 ± 5.48 pg/mL was observed (Table 38, Figure 32).

A significant fall in estrogen level to 28.67 ± 3.88 pg/mL in *Mimosa pudica* i.e Group V was observed after Letrozole treatment. The estrogen level increased to 51.83 ± 2.71 pg/mL after plant treatment for 15 days (Table 38, Figure 32).

After induction of PCOS with Letrozole in individual plant treatment group of *Symplocos racemosa* i.e Group VI, the estrogen level was found to be 30.00 ± 3.69 pg/mL. A significant increase in estrogen level up to 43.83 ± 3.19 pg/mL was recorded after plant treatment (Table 38, Figure 32).

Figure 32: Estrogen levels (pg/mL) in all the treated groups along with normal control, Letrozole Control and Natural recovery group



22.2.3 Progesterone levels

The mean plasma progesterone level in normal control group i.e Group I was observed to be 8.57 ± 0.71 ng/mL on Day 22 and 8.81 ± 0.81 ng/mL 1 indicating the stable condition.

After induction of PCOS with Letrozole treatment, the progesterone level decreased significantly to 2.16 ± 0.82 ng/mL in Letrozole control group i.e Group II (Table 38, Figure 33).

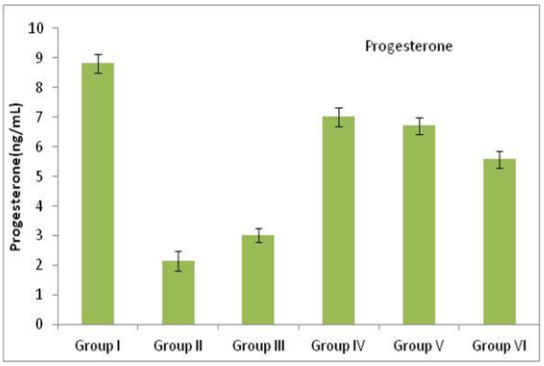
The progesterone level was observed to be 3.42 ± 0.97 ng/mL after Letrozole treatment in natural recovery group i.e Group III and after 15 days post Letrozole induction the progesterone level was found to be 3.01 ± 0.57 ng/mL (Table 38, Figure 33).

Clomiphene citrate group i.e Group IV showed decreased progesterone level as 3.45 ± 0.87 ng/mL after Letrozole treatment and an increase to 7.02 ± 0.77 ng/mL was recorded after the treatment with Clomiphene citrate (Table 38, Figure 33).

Mimosa pudica group i.e. Group V showed decreased progesterone level of 2.97 ± 0.80 ng/mL after Letrozole treatment and progesterone level increased to 6.71 ± 0.71 ng/mL after plant treatment (Table 38, Figure 33).

In *Symplocos racemosa* group i.e. Group VI, the PCOS induced rats showed decreased progesterone level of 2.80 ± 0.26 ng/mL and after plant treatment for 15 days the progesterone level was found to be 5.58 ± 0.68 ng/mL (Table 38, Figure 33).

Figure 33: Progesterone levels (ng/mL) in all the treated groups along with normal control, Letrozole Control and Natural recovery group



22.2.4 Cholesterol levels

In normal control group i.e Group I, the cholesterol level was found to be 58.00 ± 7.43 mg/dL on Day 22 and 54.83 ± 4.54 mg/dL indicating the stable condition.

In Letrozole control group i.e. Group II, the cholesterol level increased to 92.83 ± 9.54 mg/dL after the induction of PCOS with Letrozole (Table 38, Figure 34).

The animals from natural recovery group showed increase in cholesterol of 96.00 ± 5.55 mg/dL after Letrozole treatment and the cholesterol level was found to be 87.33

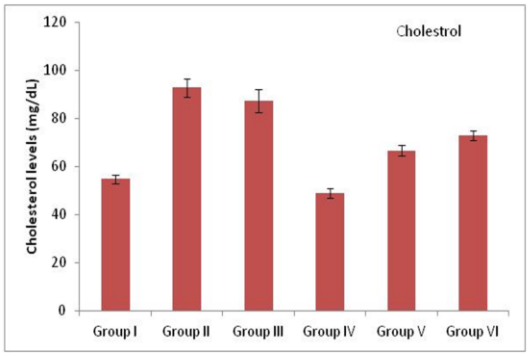
± 12.04 mg/dL after 15 days post Letrozole induction indicating no signs of recovery (Table 38, Figure 34).

In Clomiphene citrate group i.e Group IV, the cholesterol level increased to 96.83 ± 6.01 mg/dL after induction of PCOS with Letrozole. A decrease in cholesterol level of 49.00 ± 4.65 mg/dL was observed after treatment with modern drug for 15 days post Letrozole induction (Table 38, Figure 34).

The induction of PCOS with Letrozole showed increase in cholesterol level of 98.67 ± 3.88 mg/dL in *Mimosa pudica* treated group i.e Group V. A decrease in Cholesterol level to 66.67 ± 5.35 mg/dL was observed after plant treatment for 15 days (Table 38, Figure 34).

The cholesterol level in *Symplocos racemosa* treated group i.e Group VI increased to 92.50 ± 6.72 mg/dL when the animals were treated with Letrozole. After the plant treatment for 15 days, the cholesterol level decreased to 72.83 ± 4.88 mg/dL (Table 38, Figure 34).

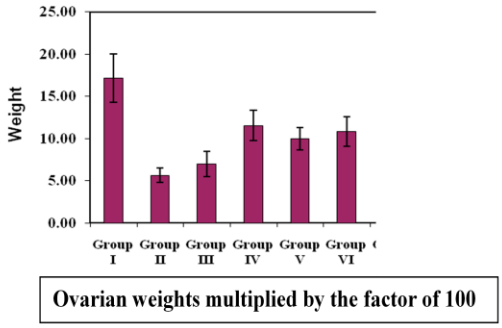
Figure 34: Cholesterol levels (mg/dL) in all the treated groups along with normal control, Letrozole Control and Natural recovery group



22.2.5 Ovarian weights (Ovary/Body weight ratio)

The ovarian weight in normal control group i.e Group I was found to be 0.14 ± 0.03 mg (Table 38a, Figure 35). The increase in ovarian weight to 0.21 ± 0.02 mg was observed in Letrozole control group i.e Group II after induction of PCOS (Table 38a, Figure 35). The ovarian weights in natural recovery group i.e Group III was found to be 0.20 ± 0.02 mg indicating no signs of recovery (Table 38a, Figure 35). The decreased ovarian weight to 0.13 ± 0.04 mg was observed in Clomiphene citrate treated group i.e Group IV (Table 38a, Figure 35). *Mimosa pudica* treated group i.e. Group V showed decreased ovarian weight of 0.16 ± 0.02 mg (Table 38a, Figure 35) and for *Symplocos racemosa* treated group i.e Group VI the ovarian weight was found to be 0.16 ± 0.01 mg (Table 38a, Figure 35).

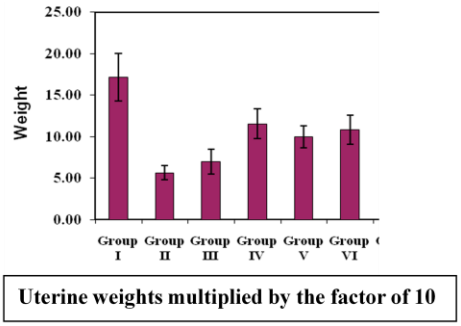
Figure 35: Ovarian weights (Ovary/body weight ratio) in all the treated groups along with normal control, Letrozole Control and Natural recovery group



22.2.6 Uterine weights (Uterus/Body weight ratio)

The uterine weight in normal rats i.e Group I was 1.71 ± 0.28 mg (Table 38a, Figure 36). The uterine weights were significantly decreased to 0.57 ± 0.09 mg (Table 38a, Figure 36). In natural recovery group i.e Group III, the uterine weight was found to be 0.70 ± 0.15 mg (Table 38a, Figure 36). After treatment with Clomiphene citrate i.e Group IV, the uterine weight was significantly increased to 1.15 ± 0.18 mg (Table 38a, Figure 36). The uterine weight in PCOS induced rat with *Mimosa pudica* treated group i.e Group V showed increased uterine weight of 1.08 ± 0.17 mg (Table 38a, Figure 36) and for *Symplocos racemosa* treated group i.e Group VI the uterine weight was found to be increased to 1.69 ± 0.24 mg (Table 38a, Figure 36).

Figure 36: Uterine weights (Uterine weight/body weight ratio) in all the treated groups along with normal control, Letrozole Control and Natural recovery group



23. Statistical significance of the results

The effect of each treatment group was evaluated statistically; Table 5.10 shows the significance of the effect of different treatments on plasma Testosterone level, Estrogen level, Progesterone level, Cholesterol level and Ovarian and Uterine weights. The plasma Testosterone level, Estrogen level, Progesterone level, Cholesterol level and Ovarian and Uterine weights for the various treatment groups were statistically compared with the corresponding levels of the Letrozole control group, Natural recovery group, Modern drug group and Normal control group. Values were represented as Mean ± SD. Data were analyzed using ANOVA followed by Dunnett's test to determine the statistical significance at ($P<0.05$, $P<0.01$ and $P<0.001$).

Table 38: Effect of various treatments on plasma Testosterone level, Estrogen level, Progesterone level and Cholesterol level in Letrozole induced PCOS rats (Mean± S.D., n=6)

Values are statistically significant at P < 0.001 when compared with Letrozole control group.

Groups	Group I Normal control	Group II Letrozole Control	Group III Natural Recovery	Group IV Clomiphene citrate	Group V *Mimosa pudica*	Group VI *Symplocos racemosa*
Testosterone (ng/dL)	245.33 ±13.84	795. 20 ± 24.10 d****	759.73 ± 55.39 a*, c****, d****	255.42 ± 17.43 a****, b****, d*	268.00 ± 18.58 a****, b****, c*, d*	279.95 ± 21.30 a****, b****, c*, d*
Estrogen (pg/mL)	55.83 ± 5.19	29.88 ± 3.64 d****	31.17 ± 7.94 a*, c****, d****	48.00 ± 5.48 a****, b****, d*	51.83 ± 2.71 a****, b****, c*, d*	43.83 ± 3.19 a****, b****, c*
Progesterone (ng/mL)	8.81 ± 0.81	2.16 ± 0.82 d****	3.01 ± 0.57 a*, c****, d****	7.02 ± 0.77 a****, b****	6.71 ± 0.71 a****, b****, c*	5.58 ± 0.68 a****, b****, c***
Cholesterol (mg/dL)	54.83 ± 4.54	92.83 ± 9.54 d****	87.33 ± 12.04 a*, c****, d****	49.00 ± 4.65 a****, b****	66.67 ± 5.35 a****, b****,	72.83 ± 4.88 a****, b***,

Comparison with Letrozole control group NS^{a*}, $P<0.05^{a**}$, $P<0.01^{a***}$, $P<0.001^{a****}$; Comparison with Natural recovery group NS^{b*}, $P<0.05^{b**}$, $P<0.01^{b***}$, $P<0.001^{b****}$
Comparison with Modern drug group NS^{c*}, $P<0.05^{c**}$, $P<0.01^{c***}$, $P<0.001^{c****}$; Comparison with Normal control group NS^{d*}, $P<0.05^{d**}$, $P<0.01^{d***}$, $P<0.001^{d****}$

Table 38a: Effect of various treatments on Ovarian and Uterine weights in Letrozole induced PCOS rats (Mean± S.D., n=6)

Groups	Group I Normal control	Group II Letrozole control	Group III Natural Recovery	Group IV Clomiphene citrate	Group V *Mimosa pudica*	Group VI *Symplocos racemosa*
Ovary/body weight ratio (mg)	0.14 ± 0.03	0.21 ± 0.02 d****	0.20 ± 0.02 a*, c****, d****	0.13 ± 0.04 a****, b****, c*, d*	0.16 ± 0.02 a***, b**, c*, d*	0.16 ± 0.01 a***, b**, c*, d*
Uterus/body weight ratio (mg)	1.71 ± 0.28	0.57 ± 0.09 d****	0.70 ± 0.15 a*, c****, d****	1.15 ± 0.18 a****, b****, c*	1.08 ± 0.17 a****, b***, b***, c*	1.69 ± 0.24 a****, b***, c****, d*

Comparison with Letrozole control group NS^{a*}, $P<0.05^{a**}$, $P<0.01^{a***}$, $P<0.001^{a****}$; Comparison with Natural recovery group NS^{b*}, $P<0.05^{b**}$, $P<0.01^{b***}$, $P<0.001^{b****}$
Comparison with Modern drug group NS^{c*}, $P<0.05^{c**}$, $P<0.01^{c***}$, $P<0.001^{c****}$; Comparison with Normal control group NS^{d*}, $P<0.05^{d**}$, $P<0.01^{d***}$, $P<0.001^{d**}$

24. Histopathology of PCOS

In 1958, Morris & Scully designed the morphological appearance of adult ovaries in 'classical' polycystic ovarian disease as consisting of bilateral enlargement and a thickening of the tunica albuginea. Although primary and secondary follicles were present in normal number, the ovarian cortex consisted of subcapsular follicular cysts representing antral follicles at different stages of atresia.

In 1960, Robert & Haines described a variety of histological patterns in "Stein-Leventhal" patients leading them to question the existence of a single syndrome.
The gross morphological heterogenicity of polycystic ovaries was also supported by Smith *et al* (1965) who reported normal appearing ovaries in 40 percent of their patients with PCOS, and no thickening of the tunica in 46 percent of those who did manifest gross ovarian enlargement.
In addition to the general cortical fibrosis, there was also fibrous replacement of the ovarian stroma with a corresponding reduction in normal stromal tissue. (Govan & Black, 1981). A comparison of 45 polycystic and normal ovaries by electron microscopy revealed the capsular thickening to be ordinary fibroplasias with collagen fibrils forming the microarchitecture. (Green & Goldzieher, 1965). The pre-ovulatory follicle in a normal ovary is surrounded by multiple layers of thecal cells, but, thecal hyperplasia occurs in majority of PCOS patients. Govan & Black (1981) noted premature leutinisation of the granulosa cells of PCO follicles. Further, these granulosa-luteal cells showed many pycnotic changes, suggesting disruption of cell growth and maturation (Shearman, 1985).

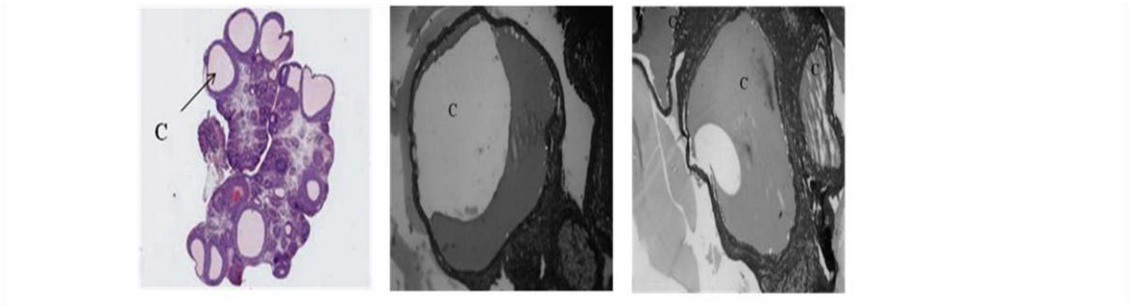


Figure 37: Section of the ovary from PCOS induced rat showing cyst with thin granulosa layer (Manneras, et al,2007: Kafali, et al, 2004)

24.1. Light microscopic observations

Advances in chemistry, physiology, immunology, and pathology—and the interactions among these fields—are essential for a better knowledge of tissue biology. The small size of cells and matrix components makes histology dependent on the use of microscopes. Under the light microscope, tissues are examined via a light beam that is transmitted through the tissue. Light microscope is one of the most common procedures in the study of tissues (Haschek and Rousseaux, 1991).

Histopathological observations:
Key to Abbreviations Used in Light Photomicrographs

PF – Primary follicle
F – Follicle
CL – Corpus luteum
A - Antrum
G – Granulosa layer
TL – Thecal layer
O – Oocyte
CO – Cumulus oophorus
CR – Corona Radiata
ZP – Zona Pellucida
AF – Atretic Follicle
C - Cyst

24.1.1 Normal Control

The histoarchitecture of the ovary shows the ovarian cortex with the presence of many follicles (F) in the various developmental stages. Each ovarian follicle consists of an Oocyte (O) surrounded by Granulosa layer (G) formed by the proliferation of follicular cells. Between the oocyte and granulosa cells, there is a layer of extracellular material called the Zona Pellucida (ZP). As the follicles grow with increasing oocyte size, the granulosa cells reorganize themselves around a larger cavity, the Antrum (A), producing follicles now called secondary or antral follicles (F). Some cells of granulosa layer form a small hillock, the Cumulus Oophorus (CO), surrounding the oocyte and protruding into the antrum. The Oocyte also becomes surrounded by granulosa cells which become the Corona Radiata (CR). The differentiation of the ovarian stromal cells around the follicle form Thecal layer (TL). A yellow body called as Corpus luteum (CL) develops in the remains of the follicle, when the secondary oocyte is liberated after bursting of the follicle. In Group I i.e Normal control, the histology of ovary was normal and intact (Figure 38, 39, 40, 41, 42).

24.1.2 Letrozole control

Ovary of Letrozole induced PCOS rats shows severely Atretic ovarian follicle (AF). Atretic secondary follicles are randomly interspread among normal follicles. In addition to marked atresia, descruption of the granulosa layer (G) is also seen. Many cysts (C) are
found. Theca layer (TL) is found to be delineating, dying cells and the debris is collected in the antrum (A) (Figure 43, 44, 45, 46, 47, 48).

24.1.3 Natural recovery

After the inducement of polycystic ovary condition in the rats by letrozole, animals of group III were left for natural recovery for 15 days before they were sacrificed. In this phase of recovery, the secondary follicles start normalizing, with the development of thecal layer (TL) and granulosa layers (G). However, the recovery is not to its potential as compared to plant and drug treatment as there were many cysts and atretic follicles (AF) still found (Figure 49, 50, 51)

24.1.4 PCOS induced rats treated with Clomiphene citrate

The ovary of the PCOS induced rats treated with Clomiphene citrate shows marked recovery in antral follicles (F) with Oocyte (O) surrounded by Cumulus oophorus (CO), Corona radiata (CR). It also shows well defined thecal layer (TL) and granulosa layer (GL). The antrum (A) appears to be clear without cell debris. The cysts are present but are very few in number as compared to Letrozole control. The ovarian cortex also shows the presence of Corpus luteum (CL) and the follicles (F) in the various stages of development (Figure 52, 53, 54, 55 56, 57, 58).

24.1.5 PCOS induced rats treated with *Mimosa pudica*

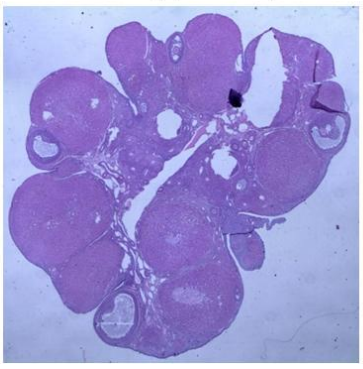
PCOS induced rats treated with *Mimosa pudica* indicates recovery of ovarian tissue. The antral follicle (F) shows the compact structure with clear antrum (A) and oocyte (O) surrounded by Granulosa cells (G), Corona radiata (CR), Cumulus oophorus (CO) and Thecal layer (TL) (Figure 59,60,61,62).

24.1.6 PCOS induced rats treated with *Symplocos racemosa*

PCOS induced rats treated with *Symplocos racemosa* shows the ovarian tissue with marked recovery of follicle (F) with intact structure of granulosa layer (G) and thecal layer (TL). It shows the presence of well developed antral follicle with oocyte (O) and antrum (A) which appears to be clear without any cell debris (63, 64, 65,66).

Normal Control Group

FIGURE 38 – Photomicrograph collage of Ovary from a control animal (H&E, X4). Note the presence of Primary follicles (PF), Antral follicles (F) and Corpus luteum (CL)



Normal Control Group

FIGURE 39 – Photomicrograph of sectioned Ovary from a control animal (H&E, X10). Note the presence of Antral follicle (F) with antral cavity (A), Oocyte (O) surrounded by Granulosa cells (G), Corona radiata (CR), Cumulus oophorus (CO) and Thecal layer (TL)

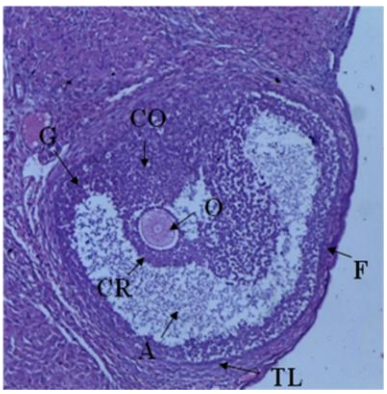


FIGURE 40 – Photomicrograph of sectioned Ovary from a control animal (H&E, X10). Arrow head indicates the presence of developing follicle (F)

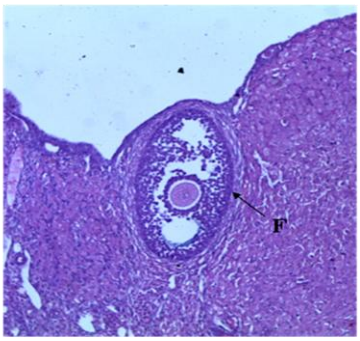


Figure 41- Photomicrograph of sectioned ovary from a control animal (H& E, X40). Note the presence of developing follicle with Oocyte (O) surrounded by Zona Pellucida (ZP), Corona radiata (CR), Granulosa cells (G) and Thecal Layer (TL)

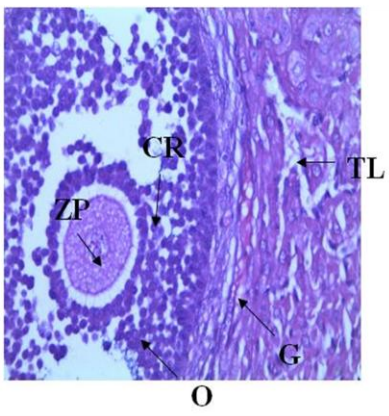
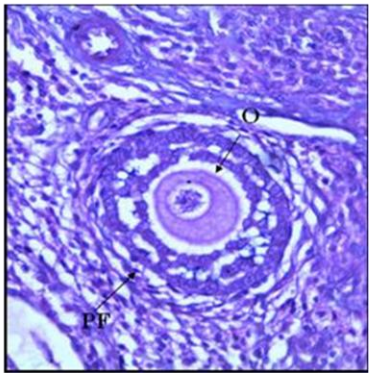
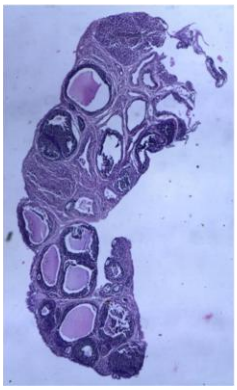


FIGURE 42 – Photomicrograph of sectioned Ovary from a control animal (H&E, X40). Arrows indicate developing Primary follicle (PF) showing the presence of Oocyte (O)



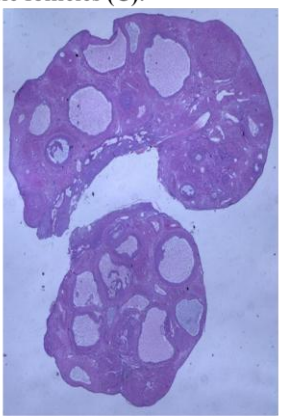
Letrozole Control Group

FIGURE 43 – Photomicrograph collage of Ovary from a rat treated with Letrozole (1.0 mg/kg) (H&E, X4). Note the presence of ovarian cortex showing the presence of Cystic follicles (C)



Letrozole Control Group

FIGURE 44 – Photomicrograph collage of Ovary from a rat treated with Letrozole (1.0 mg/kg) (H&E, X4). Note the presence of ovarian cortex showing the Cystic follicles (C).



Letrozole Control Group

FIGURE 45- Photomicrograph of sectioned Ovary from a rat treated with Letrozole (1.0mg/kg) (H&E, X10). Arrow head indicate the presence of Cystic degenerating follicle (C) with thin granulosa layer. Artretic follicle (F) with degenerated granulosa layer (G), delineated thecal layer (TL) and Antrum (A) filled with the cell debris and dying cells

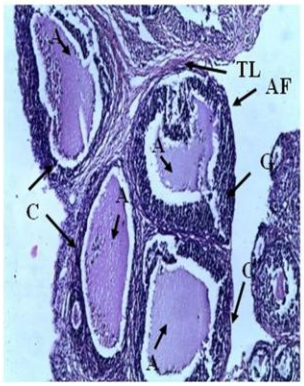
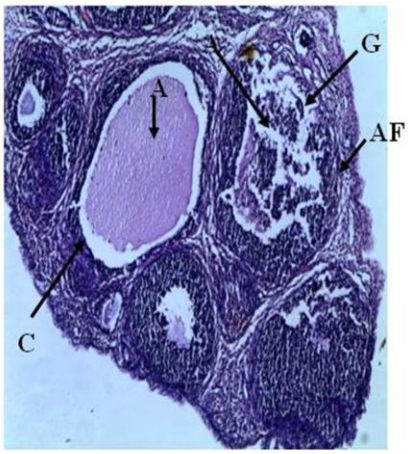


FIGURE 46- Photomicrograph of sectioned Ovary from a rat treated with Letrozole (1.0mg/kg) (H&E, X10). Note the presence of Cystic Follicle (C) with thin granulosa layer (G). Arrowheads indicate Atretic follicle (F) with Antrum (A) filled with cell debris



Letrozole Control Group

FIGURE 47- Photomicrograph of sectioned Ovary from a rat treated with Letrozole (1.0mg/kg) (H&E, X10) and the Atretic follicle (F) with degenerating granulosa layer (G)

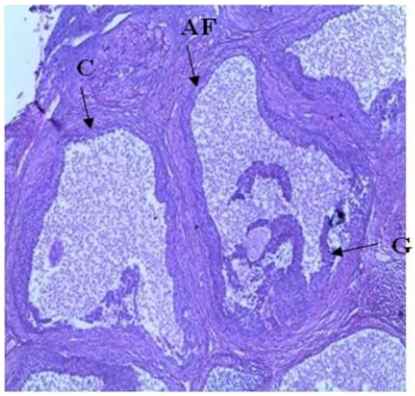
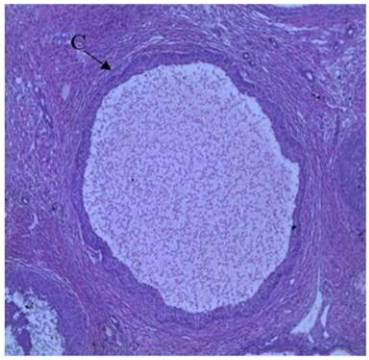
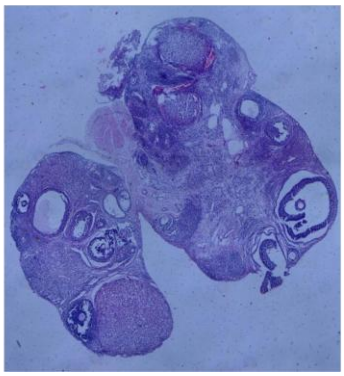


FIGURE 48- Photomicrograph of sectioned Ovary from a rat treated with Letrozole (1.0mg/kg) (H&E, X10). Note the presence of Cystic degenerating follicle (C) with scanty granulosa layer



Letrozole + Natural Recovery Group

FIGURE 49 – Photomicrograph collage of Ovary from a rat treated with Letrozole (1.0mg/kg) and left for Natural recovery (H&E, X4).
Note the presence of Atretic follicle (AF) and the developing follicle (F)



Letrozole+ Natural Recovery Group

FIGURE 50- Photo micrograph of sectioned Ovary from a rat treated with Letrozole (1.0mg/kg) and left for Natural recovery (H&E, X10). Arrow heads indicate the presence of Atretic follicle (AF) with degenerating granulosa layer (G) and delineated thecal layer (TL)

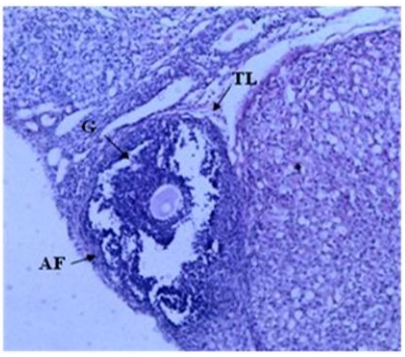
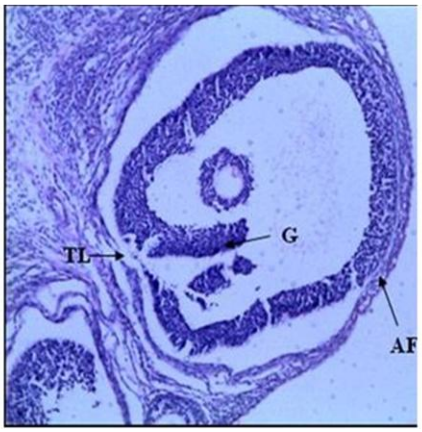
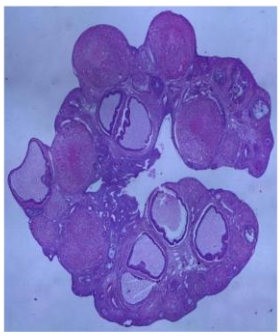


FIGURE 51- Photomicrograph of sectioned Ovary from a ra treated with Letrozole (1.0mg/kg) and left for Natural recovery (H&E,X10). Arrow heads indicate the presence of Atretic follicle (AF) with degenerating granulosa layer (G) and delineated thecal layer (TL)



Letrozole + Clomiphene citrate Group

FIGURE 52– Photomicrograph collage of Ovary from a rat treated with Letrozole (1.0mg/kg) and subsequently treated with Clomiphene citrate (H&E, X4). Ovarian cortex showing the presence of Corpus luteum (CL) and the follicles (F) in the various stages of development



Letrozole+ Clomiphene citrate Group

FIGURE 53- Photomicrograph of sectioned Ovary from a rat treated with Letrozole (1.0mg/kg) and subsequently treated with Clomiphene citrate (H&E,X10). Marked recovery showing the presence of Antral follicle (F) with intact thecal layer (TL), Granulosa layer (G) and Oocyte (O) with Corona radiata (CR) and Cumulus oophorus (CO)

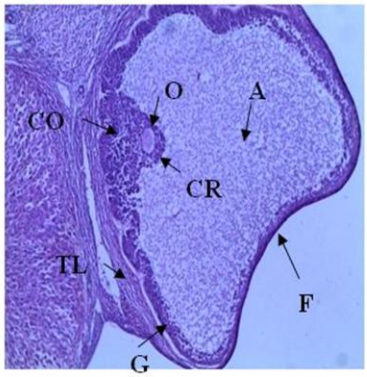
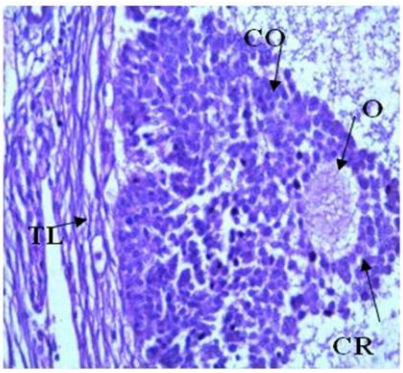
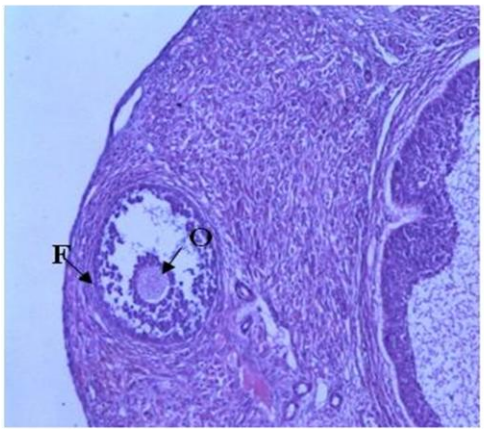


FIGURE 54- Photomicrograph of sectioned Ovary from a rat treated with Letrozole (1.0mg/kg) and subsequently treated with Clomiphene citrate (H&E, X40). Note the presence of normalized structure of follicle with intact Thecal layer (TL) and Oocyte (O) with Corona radiata (CR) and Cumulus oophorus (CO)



Letrozole+ Clomiphene citrate Group

FIGURE 55- Photomicrograph of sectioned Ovary from a rat treated with Letrozole (1.0mg/kg) and subsequently treated with Clomiphene citrate (H&E,X10). Arrow head indicate normalized structure of follicle (F) with Oocyte (O)



Letrozole+Clomiphene citrate Group

FIGURE 56- Photomicrograph of sectioned ovary from a rat treated with Letrizole (1.0 mg/kg) and subsequently treated with Clomiphene citrate (H&E, X40). Note the presence of intact structure of follicle (F) with Oocyte

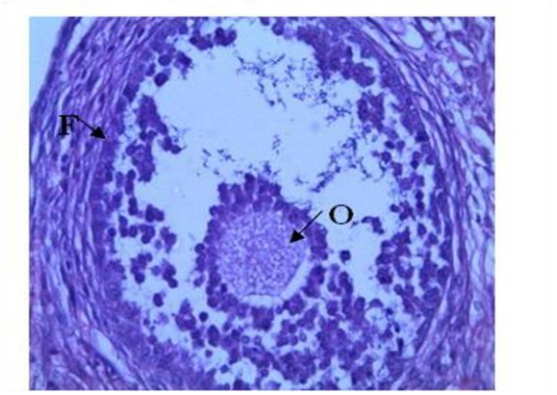


FIGURE 57– Photomicrograph of sectioned Ovary from a rat treated with Letrozole (1.0mg/kg) and subsequently treated with Clomiphene citrate (H&E, X10). Arrow heads indicate normalized structure of follicle (F) with Oocyte (O)

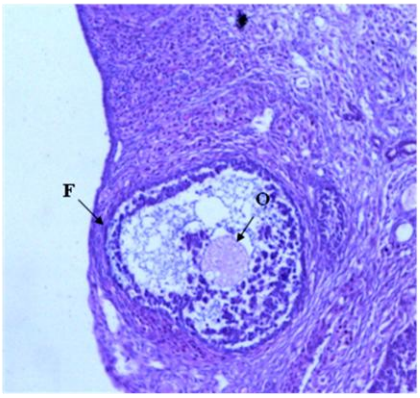
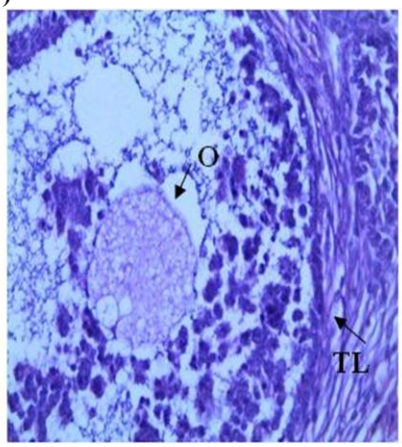
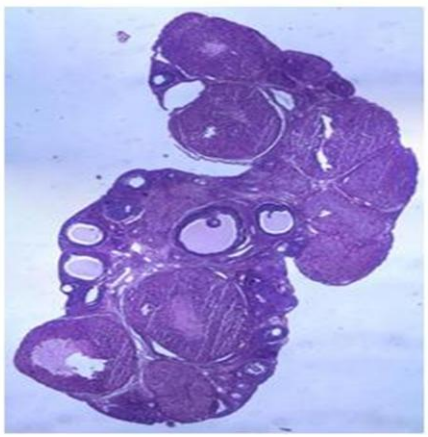


FIGURE 58- Photomicrograph of sectioned ovary treated with Letrozole (1.0 mg/kg) and subsequently treated with Clomiphene citrate (H&E, X40). Note the presence of intact structure of follicle (F) with Oocyte (O)



Letrozole+ *Mimosa pudica* Group

FIGURE 59- Photomicrograph image of ovary from a rat treated with Letrozole (1.0 mg/kg) and subsequently treated with *Mimosa pudica* (H&E,X4). Note the presence of well developed follicle (F) and Corpus luteum (CL) in the ovarian cortex



Letrozole + *Mimosa pudica* Group

FIGURE 60- Photomicrograph of sectioned ovary from a rat treated with Letrozole (1.0 mg/kg) and subsequently treated with *Mimosa pudica* (H&E, X10). Arrow heads indicate normal Antral follicle (F) wirh Oocyte (O), clear Antrum (A), intact Granulosa layer (G) and Thecal layer (TL)

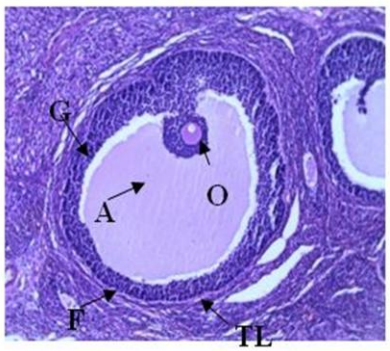


FIGURE 61- Photomicrograph of sectioned ovary from a rat treated with Letrozole (1.0 mg/kg) and subsequently treated with *Mimosa pudica* (H&E, X40). Arrow heads indicate normalized structure of follicle with Oocyte (O) surrounded by Corona radiata (CR), Cumulus oophorus (CO) and Thecal layer (TL).

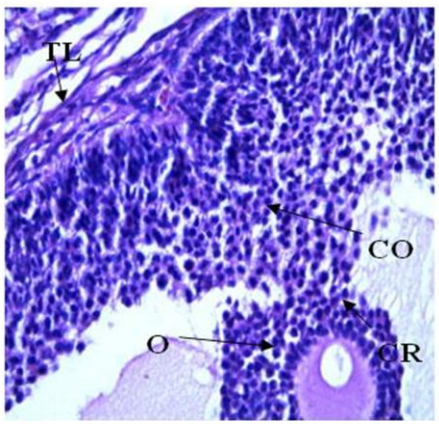
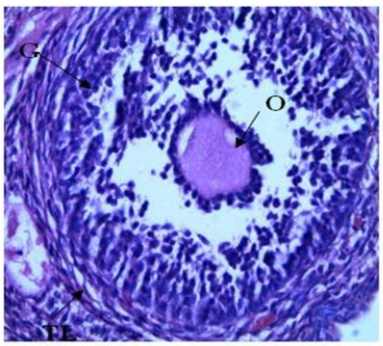
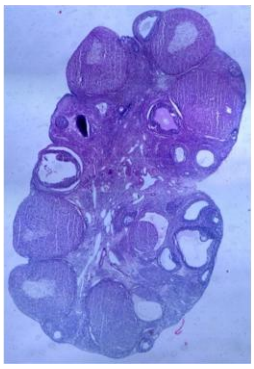


FIGURE 62- Photomicrograph of sectioned ovaary from a rat treated with Letrozole (1.0mg/kg) and subsequently treated with *Mimosa pudica* (H&E, X40) . Note the presence of normal developing follicle (F) with Oocyte (O) surrounded by Granulosa layer (G) and Thecal layer (TL).



Letrozole + *Symplocos racemosa* Group

FIGURE 63 – Photomicrograph collage of Ovary from a rat treated with Letrozole (1.0mg/kg) and subsequently treated with *Symplocos racemosa* (H&E, X4). Ovarian cortex showing the presence of developing follicles (F) and Corpus luteum (CL)



Letrozole + *Symplocos racemosa* Group

FIGURE 64- Photomicrograph of sectioned ovary from a rat treated with Letrozole (1.0 mg/kg) and subsequently treated with Symplocos racemosa Group (H&E, X10). Marked recovery showing the presence of normal antral follicle (F) with clear Antrum (A), and Oocyte (O) surrounded by Cumulus oophorus (CO), granulosa layer (G) and Thecal layer (TL)

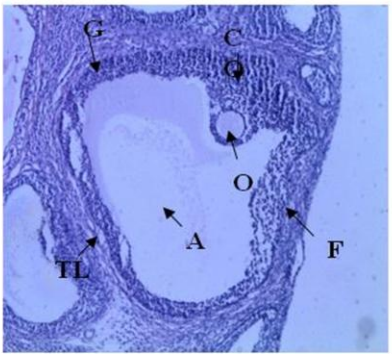


FIGURE 65- Photomicrograph of sectioned ovary from a rat treated with Letrozole (1.0 mg/kg) and subsequently treated with *Symplocos racemosa* (H&E, X40). Arrow heads indicate intact structure of follicle with Oocyte (O) surrounded by Corona radiata (CR). Cumulus oophorus (CO) and Thecal layer (TL)

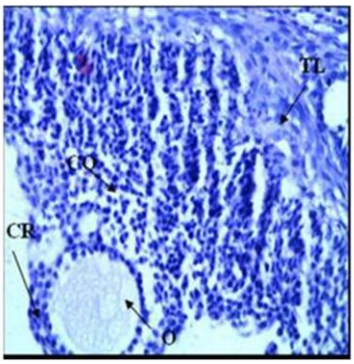
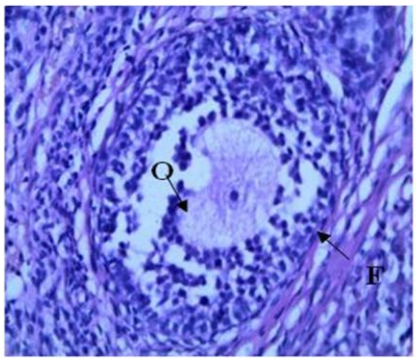


FIGURE 66- Photomicrograph of sectioned ovary from a rat treated with Letrozole (1.0mg/kg) and subsequently treated with Symplocos racemosa (H&E, X40).



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26. Electron microscopic observations

The eventual aim of biological microscopy is to observe cellular processes as they occur in living cells. Improvements in instrumentation enable researchers to observe sub cellular events as they occur in real time in a variety of different ways. There is inherent limitation of light microscopy over electron microscopy, like the lower resolving power available to examine cells. The resolving power of a microscope is a measure of how well a microscope is able to separate two points and therefore to show specimen detail. In the light microscope the smallest detectable details are, at best, in the size of about 200 nm. So, although the light microscope is an excellent tool for examining living cells in real time, it cannot give the detailed view of structures that is readily achievable by electron microscopy.

Electron microscopy (EM) is a technology ideally suited to examining small details in cells and has been in the forefront of dissecting cellular structures and their functions. It is of great value in toxicological studies, as it permits a correlation between the ultrastructural and functional changes induced by foreign chemicals (Haschek and Rousseaux, 1991).

Key to Abbreviations Used in Electron micrographs:

S- Spindle shaped Granular cells
GC- Granulosa cells
BV- Balbiani's vitelline body
B- Blood vessel
TL- Thecal layer
J- Junctional complex
Mi- Microvilli
L- Lipid droplets
F- Follicle
G- Golgi complex

26.1 Normal Control

Electron microscopic examination of the ovarian stroma shows presence of electron dense cytoplasmic granules. The spindle shaped granular cells are observed in the ovarian stromal cells. The granulosa cells of the follicle are found to have a prominent nucleus. Balbiani's vitelline body associated with the follicle is identified by the presence of dense granules, closely packed vesicles and peripheral dense fibers (Figure 67, 68, 69).

26.2 Letrozole control

The ovarian stroma of Letrozole induced PCOS rats exhibits clear signs of cell damage in granulosa cells. The altered mitochondrial structure is observed in the affected cells. The disrupted structure of mitochondria with distorted cristae is observed. The mitochondria are pyknotic, swollen or even ruptured. The endoplasmic reticulum and desmosomes are poorly developed. The nuclear chromatin material is also poorly developed in some of the granulosa cell nucleus. The chromatin margination is evident in the necrotic nucleus. There is an overall decrease in the Secretory granules (Figure 70, 71, 72, 73, 74, 75, 76).

26.3 PCOS induced rats left for Natural recovery

Under electron microscope, the ovarian stroma of the rats left for natural recovery shows the presence of desmosomes in between the cells and lamellae of collagen. The surface epithelium of the ovary shows the presence of microvilli. Prominent mitochondria and lipid droplets are seen. The stromal cells also show the presence of prominent nucleus and spindle-shaped nucleus (Figure 77, 78, 79, 80, 81, 82).

26.4 PCOS induced rats treated with Clomiphene citrate

After the PCOS rats are treated with Clomiphene citrate, the ovarian stroma shows signs of recovery when compared with Letrozole control group. The stroma shows the presence of Balbiani's vitelline bodies associated with the follicle. Prominent desmosomes are seen between the cells. Stroma shows the presence of prominent lipid droplets which are seen many in number. Surface epithelium of the ovary shows the presence of finger-like microvilli. Accumulation of collagen fibres is also observed (Figure 83, 84, 85, 86, 87, 88).

26.5 PCOS induced rats treated with *Mimosa pudica* and *Symplocos racemosa*

Post induction of PCOS with Letrozole the PCOS rats treated with *Mimosa pudica* and *Symplocos racemosa* shows prominent signs of recovery. It shows normal development of granulosa cells and thecal layer. Thecal cells show a flat and elongated shape. A large nucleus of granulosa cell with numerous dense chromatin aggregates is observed. The few lipid droplets are present in the cytoplasm. It also shows the presence of secretory granules surrounding the granulosa cells. The prominent mitochondria with normalized structure are also observed. The normal development of endoplasmic reticulum and junctional complex associated with the cells is seen. The accumulation of collagen fibers is also observed (Figure 89, 90, 91).

Normal Control Group

FIGURE 67- Electron micrograph of stromal cells of ovary from normal rat (X3000). Note the presence of spindle shaped granular cells (S); membrane bound cytoplasmic granules (bold arrow); Granulosa cells (GC) are also seen.

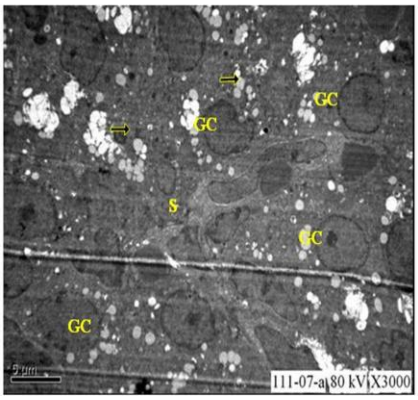


Figure 68: Electron micrograph of stromal cells of ovary from normal rat (X4000) . Note the presence of Balbiani's vitelline body (BV) beside the blood vessel (B)

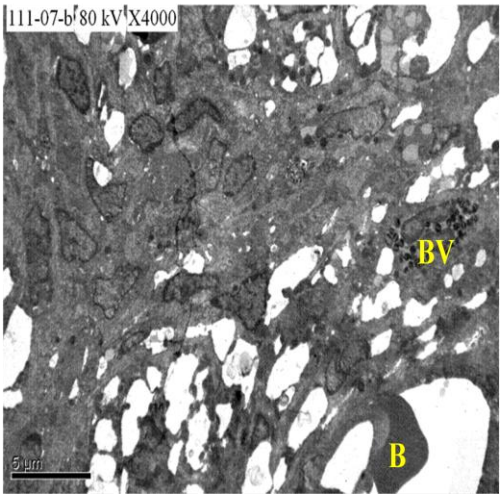
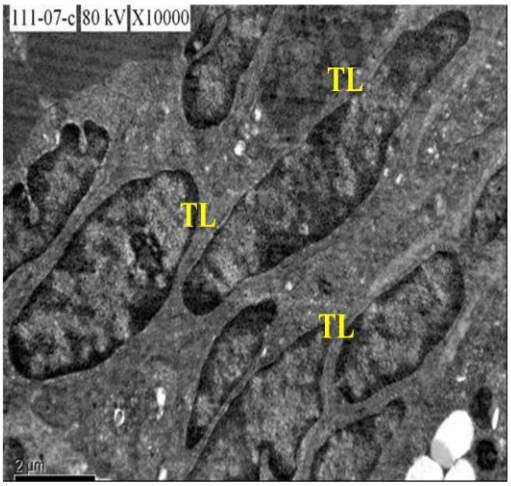


Figure 69: Electron micrograph of Ovary from normal rat (X10000) Higher power of ovarian stroma showing the presence of Thecal layer (TL) with spindle shaped thecal cells.



Letrozole Control Group

Figure 70: Electron micrograph of ovarian stroma of the rats treated with Letrozole (1.0 mg/kg) (X4000)
Note the presence of Granulosa cells (GC) in association with basement membrane (arrow heads) and Thecal layer (TL); Electron dense granules (Bold arrows); Endoplasmic reticulum (curved arrow); Mitochondria are also seen (arrows)

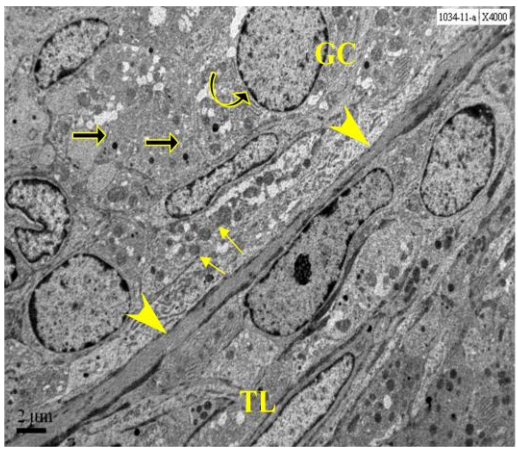


Figure 71: Electron micrograph of ovarian stroma of the rats treated with Letrozole (1.0 mg/kg) (X10000). Note the presence of Granulosa cells (GC) and dense granules (bold arrows); Endoplasmic reticulum (curved arrows) and Mitochondria are also seen (arrows)

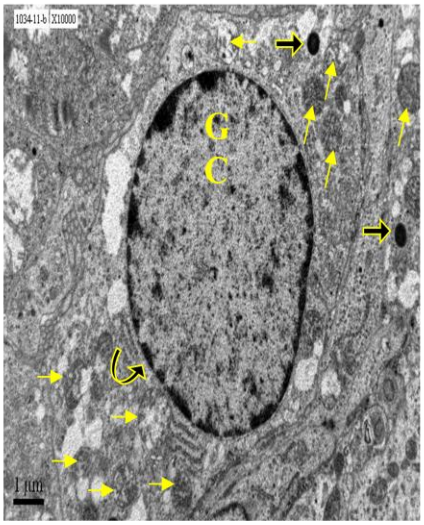


Figure 72: Electron micrograph of ovarian stroma of the rats treated with Letrozole (1.0 mg/kg) (X15000)
Note the presence of Granulosa cells (GC); Swollen and ruptured Mitochondria (arrows); Desmosomes (bold arrows) and the collagen fibers (arrow head) are also observed

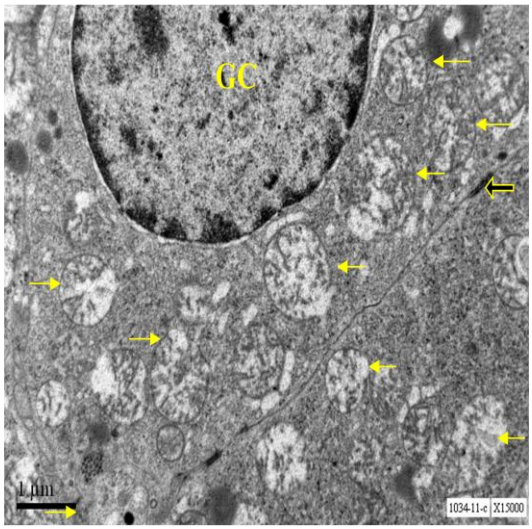


Figure 73: Electron micrograph of ovarian stroma of the rats treated with Letrozole (1.0 mg/kg) (X20000) Higher power of ovarian stroma showing the presence of Granulosa cells (GC), Swollen and ruptured Mitochondria (arrows), Endoplasmic reticulum (curved arrows), Junctional complex (J) and desmosomes (bold arrow)

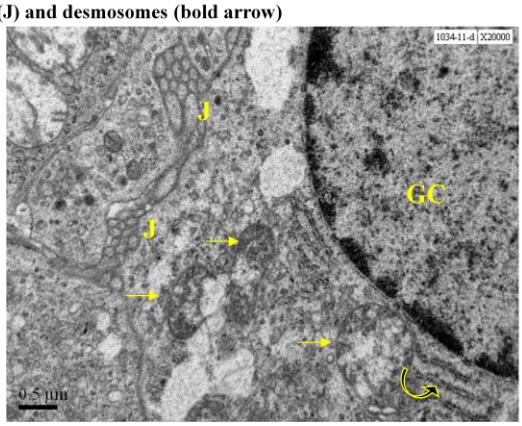


Figure 74: Electron micrograph of ovarian stroma of the rats treated with Letrozole (1.0 mg/kg) (X10000) Note the presence of Collagen fibers (arrow heads).

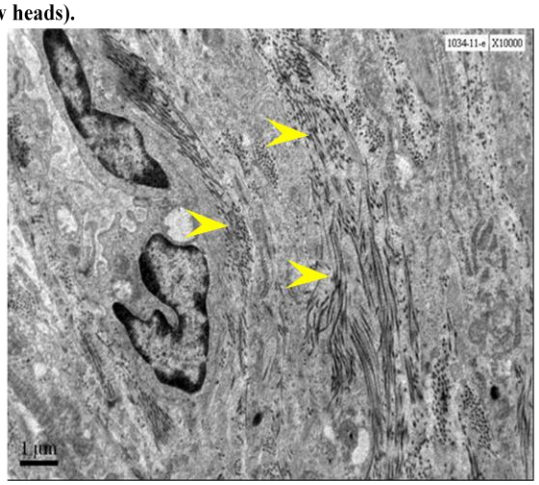


Figure 75: Electron micrograph of ovarian stroma of the rats treated with Letrozole (1.0 mg/kg) (X3000) Note the presence of Granulosa cells (GC); electron dense granules (bold arrows)

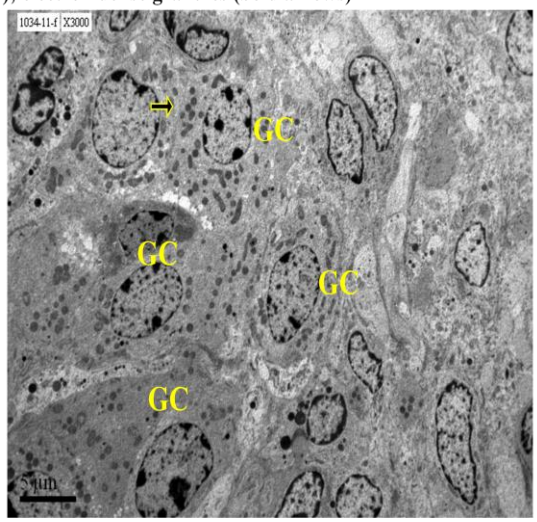
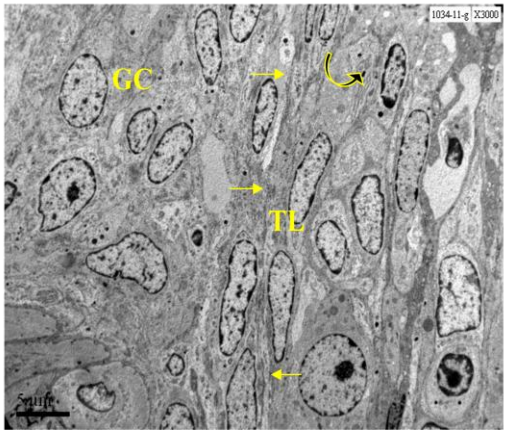


Figure 76: Electron micrograph of ovarian stroma of the rats treated with Letrozole (1.0 mg/kg) (X3000)
Note the presence of Granulosa cells (GC) associated with Basement membrane (arrows) and Thecal layer (TL); Endoplasmic reticulum (curved arrow)



Letrozole + Natural Recovery Group

Figure 77: Electron micrograph of ovarian stroma of the rats treated with Letrozole (1.0 mg/kg) and left for Natural recovery (X50000)
Note the presence of the desmosome (bold arrow) and collagen in lamellae (arrow head)

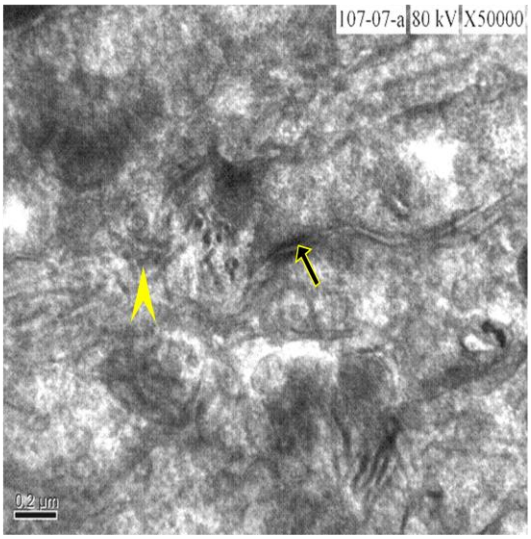


Figure 78: Electron micrograph of ovarian stroma of the rats treated with Letrozole (1.0 mg/kg) and left for Natural recovery (X20000). Note the presence of desmosome (bold arrow) associated with the different follicles in the stroma of the ovary and lamellae of collagen (arrow heads)

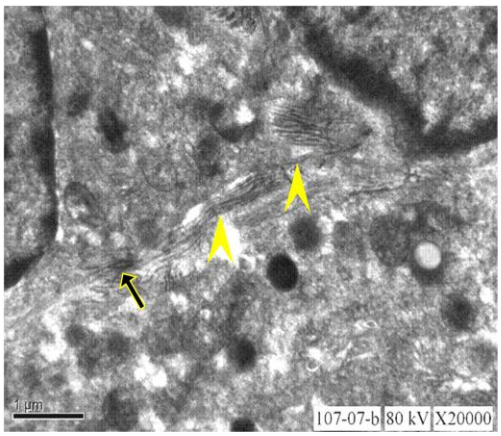


Figure 79: Electron micrograph of ovarian stroma of the rats treated with Letrozole (1.0 mg/kg) and left for Natural recovery (X3000) Note the presence of Granulosa cells (GC) and electron dense granules (bold arrows)

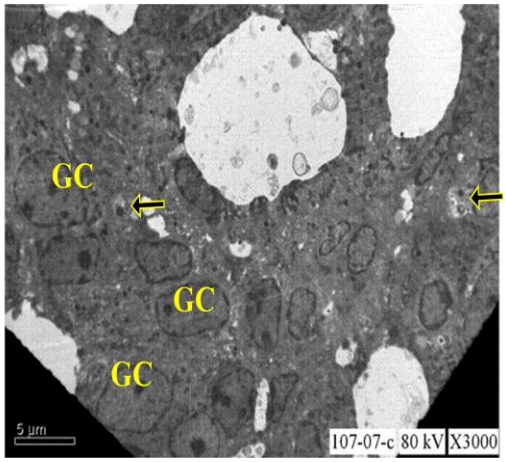


Figure 80: Electron micrograph of ovarian stroma of the rats treated with Letrozole (1.0 mg/kg) and left for Natural recovery (X30000) Note the presence of microvilli (Mi) of the ovarian surface epithelium and shows the lamellae of collagen (arrow head)

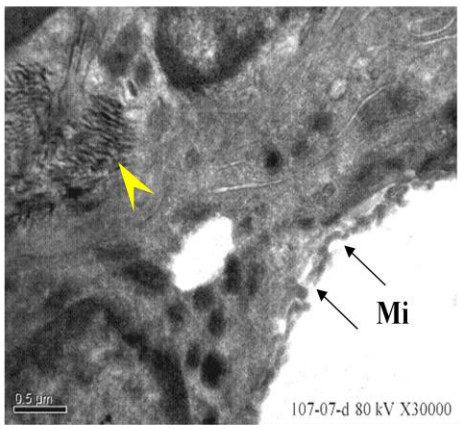


FIGURE 81- Electron micrograph of ovarian stroma of the rats treated with Letrozole (1.0mg/kg) and left for Natural recovery (X3000). Note the presence of mitochondria (arrows); lipids droplets (L) and Granulosa cells (GC)

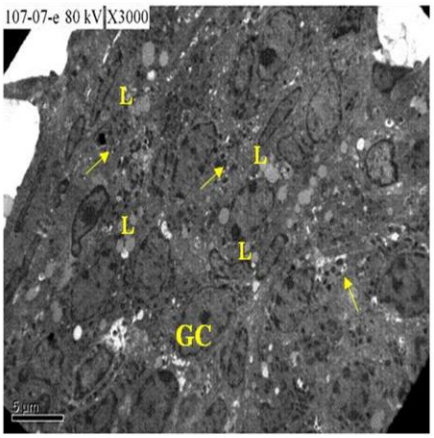
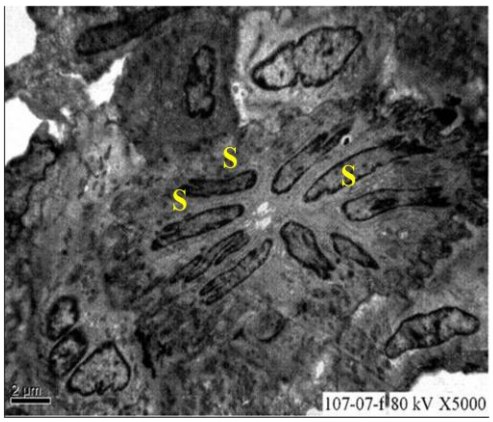


Figure 82: Electron micrograph of ovarian stroma of the rats treated with Letrozole (1.0 mg/kg) and left for Natural recovery (X5000) Stromal cells showing the presence of spindle-shaped granular cells (S)



Letrozole + Clomiphene citrate Group

Figure 83: Electron micrograph of ovarian stroma of the rats treated with Letrozole (1.0 mg/kg) and subsequently treated with Clomiphene citrate (X2500) Stroma shows the presence of prominent Balbiani's vitelline body (BV)

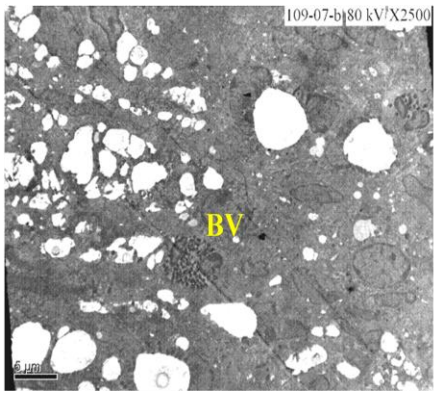


Figure 84: Electron micrograph of ovarian stroma of the rats treated with Letrozole (1.0 mg/kg) and subsequently treated with Clomiphene citrate (X15000) Association of the follicle (F) and the Balbiani's vitelline body (BV)

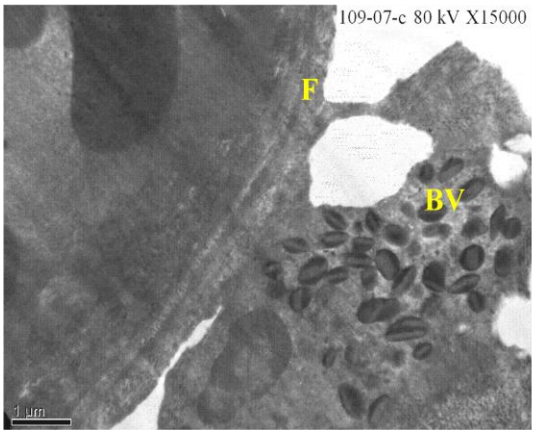


Figure 85: Electron micrograph of ovarian stroma of the rats treated with Letrozole (1.0 mg/kg) and subsequently treated with Clomiphene citrate (X20000). Note the presence of desmosome (bold arrow) and collagen accumulation (arrow head)

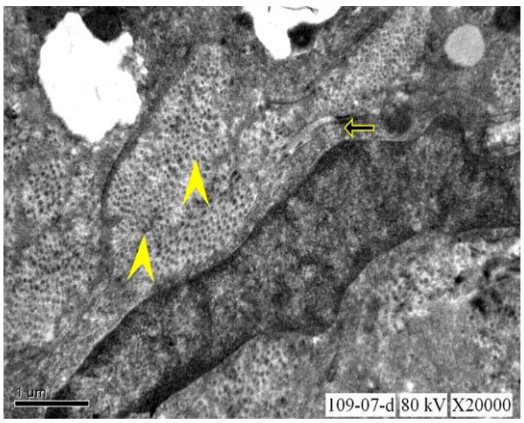


Figure 86: Electron micrograph of ovarian stroma of the rats treated with Letrozole (1.0 mg/kg) and subsequently treated with Clomiphene citrate (X3000) Stroma shows the presence of lipid droplets (L)

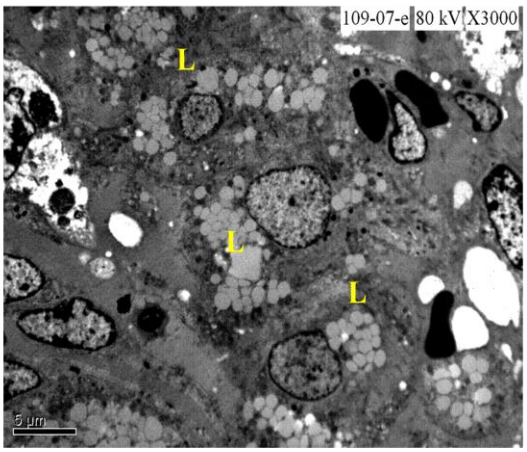


Figure 87: Electron micrograph of ovarian stroma of the rats treated with Letrozole (1.0 mg/kg) and subsequently treated with Clomiphene citrate (X10000) Note the presence of Collagen fibers (arrow head) and microvilli (Mi) and lipid droplets (L) are also seen

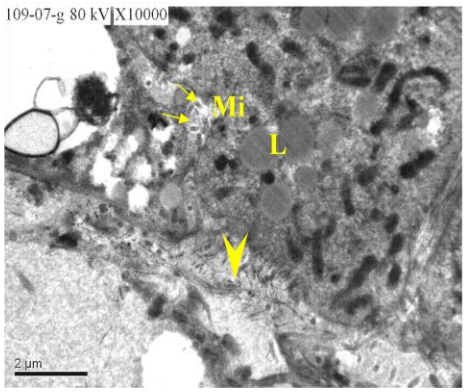
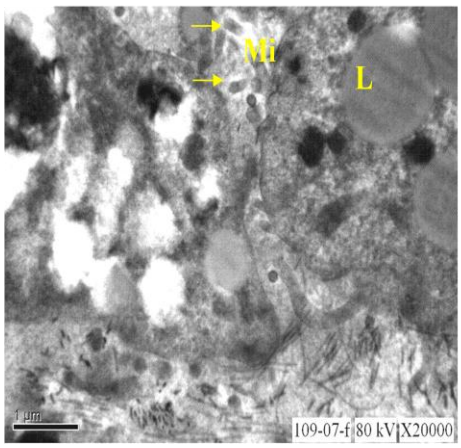


Figure 88: Electron micrograph of ovarian stroma of the rats treated with Letrozole (1.0 mg/kg) and subsequently treated with Clomiphene citrate (X20000). The surface epithelium of the ovary shows the presence of microvilli (Mi); Prominent lipid droplets (L) are also seen



Letrozole + *Mimosa pudica* + *Symplocos racemosa* Group

Figure 89: Electron micrograph of ovarian stroma of the rats treated with Letrozole (1.0 mg/kg) and subsequently treated with *Mimosa pudica* + *Symplocos racemosa* (X3000)
Note the presence of Thecal layer (TL); The prominent Lipid droplets (L) are also seen

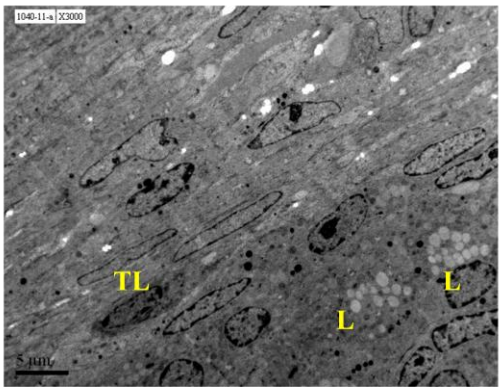


Figure 90: Electron micrograph of ovarian stroma of the rats treated with Letrozole (1.0 mg/kg) and subsequently treated with *Mimosa pudica* + *Symplocos racemosa* (X2500)
Note the presence of Granulosa cells (GC) surrounded by the Secretory granules (bold arrows); Mitochondria (arrow) are also seen

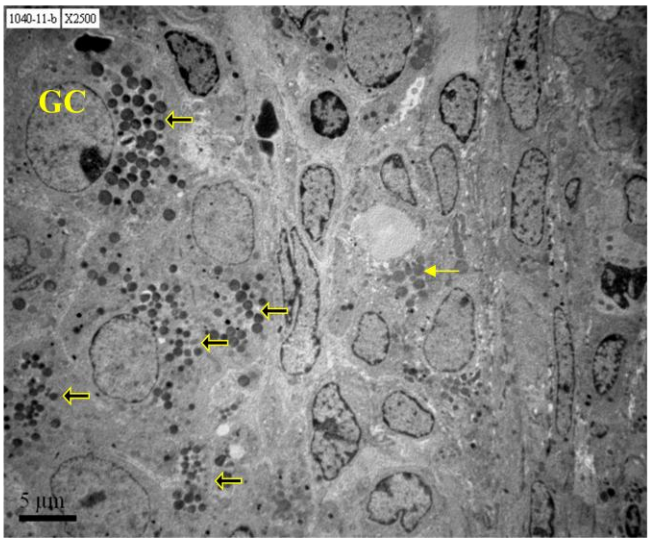
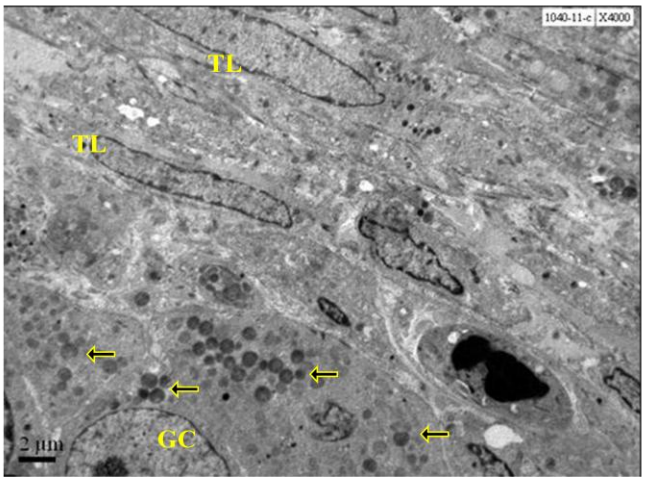


Figure 91: Electron micrograph of ovarian stroma of the rats treated with Letrozole (1.0 mg/kg) and subsequently treated with *Mimosa pudica* + *Symplocos racemosa* (X4000)
Note the presence of Thecal layer (TL) and Granulosa cells (GC) surrounded by the Secretory granules (bold arrows)



27. Discussion

The major cause of female infertility in recent years is polycystic ovary syndrome (PCOS). Several factors contribute to the difficulties in diagnosis of PCOS. Presenting signs and symptoms of PCOS are heterogeneous and vary over time, in addition, a precise and uniform definition of this syndrome is lacking. The definite underlying mechanism of PCO formation remains unknown (Lee *et al*, 2003). The resultant complex disease of polycystic ovary syndrome (PCOS) in women involves anovulation, hyperandrogenism, obesity and insulin resistance. The anovulation is associated with a disturbing feedback system by ovarian estrogens to the hypothalamus, resulting in a high elevated luteinizing hormone concentration that is detrimental to follicular growth (Ehrmann, 2005; Desai *et al*, 2012).

The world's oldest known system medicine is attracting a legion of new patients suffering from a modern epidemic infertility. At present, the available drugs used in the management of PCOS are clomiphene citrate, gonadotropin, troglitazone, metformin and oral contraceptive pills. All these allopathic medicines display various side effects such as headache, gastrointestinal effects (nausea, diarrhea, flatulence) at initiation of therapy, weight loss, taste disturbances, respiratory infection, abdominal discomfort, muscle and joint pain, constipation, vaginal bleeding or irritation and psychological effects such as mood swings, depression and bloating. Due to adverse effects caused by the modern medicines, alternative medicines like herbal medicines are gaining importance (Sasikala *et al*, 2010).

In the present research work, polycystic ovary was induced in the rats by the oral administration of a chemical, letrozole. The animals were then treated with *Mimosa pudica* and *Symplocos racemosa*. The efficacy was evaluated and compared with modern drug Clomiphene citrate. The plants used in the present research work *Mimosa pudica* and *Symplocos racemosa* have been evaluated for all their activities but, there is no literature survey on these plants for the treatment of PCOS.

Numerous experimental models for PCOS have been developed in rats. It has been reported that the hormonal substances such as estradiol valerate, dehydroepiandrosterone and neonatal androgenization have been employed to induce PCOS (Mahajan, 1988). In spite of their effectiveness, all these hormonal treatments

cause a sudden appearance of polycystic ovaries due to disturbances in the metabolic and physiologic processes. Thus, these models do not reproduce exactly what happens in the real syndrome (Baravalle *et al*, 2006). Therefore despite the availability of numerous animal models for the study of human PCOS, a fully convincing model as a whole has not been established to date (Kafali *et al*, 2004). In estradiol valerate rat model, although anatomy and physiology of the ovary resemble those of human PCOS, progressive degeneration of hypothalamus and altered presence of pituitary to LHRH rendered this model inappropriate for studying hypothalamus pituitary axis in PCOS. Dehydroepiandrosterone (DHA) model showed many features of PCOS but continuous administration of DHA and its metabolites interfered in many experimental conditions (Mahajan, 1988). In neonatally androgenized female rat model, ovaries were smaller than those of controls and had normal tunica albuginea and no hyperthecosis. Moreover, because testosterone was administered prior to differentiation of hypothalamus and pituitary cells these organs were rendered nonresponsive to steroids, luteinizing hormone-releasing hormome (LHRH), and FSH. Although anatomic features consequent to PCOS resulted from constant-light similar to human PCOS and ovarian cells still retained the ability to respond to FSH and LHRH, no increment in levels of LH and androgens that have a pivotal role in PCOS rendered this model different from human PCOS (Mahajan, 1988).

Polycystic ovaries may be created de novo as a result of exogenous androgen administration or secondarily to endogenous androgen excess (Azziz *et al*, 1994). Abnormal follicular maturation or acceleration of follicular atresia was reported in presence of elevated intraovarian androgen levels. Therefore, intraovarian androgen excess resulting from either circulating hyperandrogenemia or abnormal steroidogenesis may result in abnormal follicular development and polycystic ovary. Testosterone and androstenedione were converted into estradiol and estrone, respectively, by P450 aromatase, which was expressed in ovary. A decrease in activity of this enzyme could be expected to result in increased ovarian androgen production and development of PCOS (Kafali *et al*, 2004).

In the present study, the animal model with PCOS was developed using non-steroidal aromatase inhibitor letrozole in rat which showed many histologic and biochemical findings consistent with human PCOS and the efficacy of *Mimosa pudica* and

Symplocos racemosa was evaluated by treating Letrozole induced PCOS rats with these plants. The endocrine disturbances similar to those in human PCOS were observed, but the metabolic characteristics of PCOS were not investigated in the present study. The induced PCOS was confirmed through polycystic ovarian histopathology and hormone profiles.

In polycystic ovary syndrome, the testosterone levels increase in humans (Michelmore *et al*, 1999; Bhattacharya *et al*, 2005; Sridhar and Nagamani, 2006; Homburg, 2009; Kafali *et al*, 2004; Baravalle *et al*, 2006). The similar results were obtained in the present research work. In the present research work, when the rats were induced with PCOS by letrozole, the testosterone levels were found to be significantly increased when compared with normal rats. These results of the current work also coincide with the reports by Desai *et al*, 2012, Sasikala and Shamila, 2009 and Maharjan *et al*, 2011. The similar effect with treatment of Asokarishtam has been reported (Sasikala and Shamila, 2009). Desai *et al*, 2012 have also shown the significant decrease in testosterone levels after the treatment of PCOS induced rats with *Aloe barbadensis* Mill. formulation. This decrease in hyperandrogenism was also observed by Maharjan *et al*, 2011.

A deficiency in the activity of the aromatase enzyme is one of the many intra-ovarian disturbances thought to cause androgen excess in PCOS. Aromatase catalyzes the rate limiting step in the biosynthesis of estrogens from androgens. Letrozole is a non-steroidal inhibitor of the aromatase enzyme, which results in androgen excess and promotes development of PCOS (Desai *et al*, 2012; Kafali *et al*, 2004; Manneras *et al*, 2007). Therefore PCOS is manifested primarily by anovulation and hyperandrogenism. The chronic anovulation is seen as a result of an increase in the LH/FSH ratio. An increase in LH concentration PCOS is seen due to inappropriate estrogen feedback to the pituitary (Kafali *et al*, 2004).

Earlier findings, have reported that being a non-steroidal aromatase inhibitor Letrozole blocks the conversion of testosterone to estradiol. This leads to the reduction in estrogen production (Kafali *et al*, 2004; Maharjan *et al*, 2011; Manneras *et al*, 2007; Sasikala and Shamila, 2009). Similar effect was seen in the present work that the plasma estrogen levels were found to be significantly decreased when the

animals were treated with Letrozole for the induction of PCOS. When the animals were treated with *Mimosa pudica* and *Symplocos racemosa* the estrogen levels were returned to the normalcy and they were found to be comparable to those in normal control group. This is in accordance with earlier findings with the treatment of Asokarishtam (Sasikala and Shamila, 2009). The significant increase in estrogen levels were also observed by maharjan *et al*, 2011 when the PCOS induced rats were treated with *Aloe barbadensis* Mill. formulation.

In the present research work, the plasma progesterone levels decrease significantly after induction of PCOS with Letrozole. This is in accordance with the earlier observations (Baravalle *et al*, 2006; Kafali *et al*, 2004; Sasikala and Shamila, 2009). Sasikala and Shamila, 2009 have shown the elevated progesterone levels when the PCOS induced rats were treated with Asokarishtam. The similar effect was seen in the current work, when the animals were treated with *Mimosa pudica* and *Symplocos racemosa* and it was found to be comparable to those in normal control group.

Women with PCOS are hyperandrogenemic which is associated with alterations in circulating lipids and lipoprotein levels resulting in dyslipidemia (Coviello *et al*, 2006; Desai *et al*, 2012; Legro *et* al, 2001; Sam *et al*, 2005). Characteristically PCOS patients have elevated cholesterol levels (Desai *et al*, 2012; Sasikala and Shamila, 2009). PCOS has been associated with increased prevalence of lipid related abnormalities including reduced high density lipoprotein (HDL) cholesterol and increased low density lipoprotein (LDL) cholesterol and triglyceride concentrations (Coviello *et al*, 2006; *Desai et al*, 2012; Lo *et al*, 2006). While hyperandrogenism is likely to play some role in these abnormalities, hyperinsulinemia (insulin resistance) appears to be the most important contribution to these abnormalities. These abnormalities are known to be highly predictive of cardiovascular disease (Legro, 2003; Carmina and Lobo, 1999; Paradisi *et al*, 2001). Hypertension is extremely prevalent, particularly in older women with PCOS and those who are obese. Women with PCOS have a 7-fold increased risk of myocardial infarction. Coronary disease is more prevalent in women with PCOS (Carmina and Lobo, 1999). Bhattacharya (2005), Banaszewska (2006) and Barber (2006) and their coworkers have observed that people with polycystic ovary syndrome tend to be obese, probably due to high lipid and cholesterol content. The similar effect was seen in the current research work

after the induction of PCOS. The PCOS induced rats showed elevated cholesterol levels which decreased significantly when the PCOS induced rats were treated with *Mimosa pudica* and *Symplocos racemosa*. These cholesterol levels were found to be comparable to those in normal control group. This is in accordance with the earlier reports by Sasikala and Shamila, 2009 when the PCOS induced rats were treated with Asokarishtam. Desai *et al*, 2012 have also reported the decrease in cholesterol level when the PCOS induced rats were treated with *Aloe barbadensis* Mill. formulation.

The administration of *Mimosa pudica* and *Symplocos racemosa* along with Clomiphene citrate also exerted the significant effect in the normalization of estrogen levels, progesterone levels and cholesterol levels.

In the present research work, when the rats were induced with PCOS by Letrozole, there was a significant increase in ovarian weight when compared with Letrozole control group. This is in accordance with the earlier findings (Desai *et al*, 2012; Maharjan *et al*, 2011; Manneras *et al*, 2007; Sasikala and Shamila, 2009). Similar observation has also been made in PCOS induced guinea pig after the treatment with Estradiol – 171 (Quandt and Hutz, 1993). Schulster *et al*, 1984 have also reported increased ovarian weights after the induction of PCOS with Estradiol Valerate. In the present work when *Mimosa pudica* and *Symplocos racemosa* were given to the PCOS induced rats the ovarian weights decrease significantly when compared with letrozole control group.

In earlier studies conducted on rats, it was seen that there was decrease in uterine weight after the induction of PCOS with Letrozole (Kafali *et al*, 2004). In the current study, the similar effect was seen after the treatment with letrozole. The uterine weights were returned to the normalcy when the treatment of *Mimosa pudica* and *Symplocos racemosa* was given. In the PCOS induced rats given repeated treatment of *Mimosa pudica* and *Symplocos racemosa* also showed the significant recovery of uterine weight.

The biochemical results are also supported by Histopathological observations of both light microscopy and electron microscopy in the normal control, letrozole control and the various treatment groups. It is reported that the histopathological study of PCOS induced rats show the formation of cysts in the ovary (Baravalle *et al*, 2006; Kafali *et*

al, 2004). The ovarian cortex shows the presence of Atretic follicles and the formation of more than two cysts in the ovary. The cysts show the attenuated layer of granulosa cells and hyperplasia of thecal layer. The degeneration is observed in antral follicles of all sizes with degeneration in granulosa cell layer (Brawer *et al*, 1986; Kafali *et al*, 2004; Schulster *et al*, 1984). Brawer *et al*, 1986 and Sasikala and Shamila, 2009 have shown that the Atretic follicles exhibit massive degeneration and sloughing - off of the central granulosa layer into the antrum. Thus the follicles become atretic with the presence of dying cells and debris in the antrum. In PCOS condition the corpora lutea do not form or the number of corpora lutea are diminished indicating anovulation and the frequency of estrus cycle is almost nil in PCOS rats (Brawer *et al*, 1986; Sasikala and Shamila, 2009). De Leo and coworkers, 1998 have reported that in PCOS high local androgen concentrations are responsible for anovulation by direct effect on the ovary. Androgen-induced follicular atresia is thought to occur by entry of androgens into the granulosa layer of pre-antral follicles, where they bind to the cell receptors and cause the cell death. Androgens cause deterioration of follicles by increasing the number of pycnotic granulosa cells and degenerating oocytes. The similar observations were seen in the current research work when the rats were induced with PCOS by Letrozole. Following the administration of Letrozole, the ovarian cortex showed the presence of definitive cysts which are particularly prominent with thin granulosa layer. Much of the ovary was occupied by the large, often severely atretic follicles. No corpora lutea were observed which was the indicative of absence of estrus and absence of ovulation. The granulosa layer of the follicle underwent degeneration and it also showed the presence of delineated thecal layer. The antrum of the follicle also became filled with the dying cells and cell debris. Decrement in number of primary and secondary follicles was also noted.

The histopathalogical observations of the treated group i.e *Mimosa pudica* and *Symplocos racemosa* and in treatment with Clomiphene citrate showed marked recovery of the ovarian tissue with the presence of normalized structure of antral follicle. The light microscopic observations also revealed the presence of many well defined antral follicles in the process of normalizing. The follicles showed normal granulosa and defined thecal layers. The follicles also showed the presence of a clear antrum free of any cell debris. The cyst observed was not more than one. The

presence of corpora lutea was also seen after plant treatment. The ovarian cortex also showed the presence of many follicles in the various stages of development.

Ultrastructural observations of the ovarian stroma in Letrozole induced PCOS rats have shown significant cytoarchitectural changes in the cells. Electron microscopy of the ovary treated with letrozole revealed the damage to the granulosa cells. The cell organelles such as mitochondria, endoplasmic reticulum and golgi complex were also poorly developed with the absence of secretory granules. These observations indicate that the ovary is affected by polycystic condition. When the sections of the ovary from rats left for natural recovery were scanned by electron microscope, the stroma showed the presence of prominent desmosomes. The surface epithelium showed the presence of finger-like microvilli. Stromal cells showed the presence of prominent mitochondria, nucleus, spindle-shaped nucleus and lipid droplets. Lipid droplets were seen more as compared to the ovary treated with plant and drug, suggesting less recovery of animals when they were left for natural recovery. After treatment of the animals with the clomiphene citrate, the stroma showed the presence of prominent Balbiani's vitelline bodies associated with the follicles. Desmosomes were seen in between the cells. Surface epithelium showed the presence of microvilli. Accumulation of collagen fibers was observed. Stroma also showed the presence of lipid droplets but, was less as compared to natural recovery and more as compared to plant treatment.

After treatment of the animals with the Clomiphene citrate, the stroma showed the presence of prominent Balbiani's vitelline bodies associated with the follicles. Desmosomes were seen in between the cells. Surface epithelium showed the presence of microvilli. Accumulation of collagen fibers was observed. Stroma also showed the presence of lipid droplets but, was less as compared to natural recovery and more as compared to plant treatment. When the PCOS rats were given the treatment of *Mimosa pudica* and *Symplocos racemosa*, follicles started recovering with normal development of granulosa cells with associated basement membrane and thecal layer. The desmosomes associated with the granulosa cells were also normal. The secretory granules were observed surrounding the granulosa cells. Important cell organelles like mitochondria, endoplasmic reticulum and golgi complex were prominently seen. They were many in number as compared to natural recovery and clomiphene citrate

treatment suggesting good recovery of the animals. Few lipid droplets were also seen. However, these droplets were comparatively less than natural recovery and drug treatment.

In the present investigation, whole plant of *Mimosa pudica* and stem bark of *Symplocos racemosa* significantly restored blood biochemical parameters such as testosterone, estrogen, progesterone and cholesterol levels. It also restored the histology of ovarian tissue. When these biochemical and histopathalogical observations were compared with clomiphene citrate group, *Mimosa pudica* and *Symplocos racemosa* was found to be efficacious in the management of PCOS.

28. Conclusion

The present investigation shows that the hallmark of the endocrine disorder of PCOS is hyperandrogenism and anovulation. The present study shows that the increased testosterone levels, decreased estrogen and progesterone levels, degenerated structure of follicles along with the presence of cysts in the ovarian tissue are apparent in Letrozole induced PCOS rats. The polyherbal combination exhibits significant recovery of testosterone, estrogen, progesterone levels and ovarian tissues. The plants probably show good anti-androgen effect by reducing increased androgen levels. The plants also show the good estrogenic effect. The plants also provide the protection against obesity by reducing the total cholesterol levels. The anti-androgen and estrogenic effects of the plants prevent ovarian cells dysfunction in PCOS and improve fertility. The composite slurry of plant material evaluated in the current study provides a similar response by significantly lowering the plasma testosterone and total plasma cholesterol levels. The plant treatment also shows increase in the plasma estrogen and progesterone levels along with the significant recovery of ovarian and uterine weights. The statistical treatment of the data on testosterone, estrogen, progesterone, total cholesterol levels, ovarian weights and uterine weights indicate significant recovery of animals treated with plants. The biochemical changes are also supported by histopathological (Light and Electron microscopy) observations which support the recovery of the ovarian tissue. The changes observed under different magnifications in microscopy reveal the recovery occurring at the cellular level. The observations support the potential use of the plants combinative therapy with modern drug in the management of PCOS. The light and electron microscopy observations for

the plants shows improved cytoarchitecture. There is minimum hypertrophy of cell organelles and increase in secretory granules.

Based on the biochemical and histopathological evaluation, it can be concluded that the *Mimosa pudica* and *Symplocos racemosa* exert significant effect in the recovery of PCOS.

The model used in the current research work for the study of PCOS proved to be a good model due to reasons :- i) Elevated testosterone levels ii) Histopathological changes as seen in human PCOS iii) Shorter treatment period for induction of PCOS iv) Easy inducement of PCOS by chemical (oral administration) v) Consistent response of the model to various treatments. However, this model used in the present research work has been reported to have drawbacks like failure to bring about elevation of luteinizing hormone level (LH) as seen in PCOS of human. Despite this drawback, the model and study design demonstrates the feasibility of evaluating herbal remedies for PCOS management.

Findings of the current study can provide base line data for designing further investigations on the therapeutic benefits of *Mimosa pudica* and *Symplocos racemosa* in the management of PCOS. So that such plants or combination of these plants can be prescribed for concomitant administration with modern drug so as to reduce the side effects of modern drug without compromising the therapeutic activity. This can be extrapolated to humans, but, needs further confirmation of the concept. Further, the dosage and treatment regimen needs modification and further rationalization by conducting suitably designed clinical trials.

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30. BIOAVAILABILITY:

Pharmacokinetics is the study and characterisation of the time course of drug absorption, distribution, metabolism and excretion (ADME) and the relationship of these processes to the intensity and time course of therapeutic and toxicologic effects of drugs. In general pharmacokinetic parameters are derived from the measurement of drug concentrations in blood or plasma (Gibaldi, 2005; Smith *et al*, 2001). It is concerned with the effects the body has on the medicine and specifically the concentrations, which can be achieved at active sites. Pharmacokinetics is the science of quantitative actions between a biological organism and pharmacology within it. In practice this discipline is applied mainly to drug substances, though in principle it concerns itself with all manner of compounds residing within an organism or system, such as nutrients metabolites, endogenous hormones, toxins, etc. So, in basic terms, while pharmacodynamics explores what a drug does to the body and looks at the effects of an agent at active sites in body, pharmacokinetics explores what the body does to the drug (Meibohm, 2006; Kallen, 2008; Jambhekar and Breen, 2009). Pharmacokinetic information about herbal medicines is not widely available due to the several factors including lack of studies and inadequate reporting.

Pharmacokinetic data is important for understanding the interactions between herbs and the biological systems. For a given dose of any herbal medicine, its physiological effect (or that of its constituents) will be governed by the effective tissue concentration of the medicine which in turn is determined by pharmacokinetic parameters – the absorption, distribution, metabolism and excretion of its various components. The pharmacokinetic study of herbal medicine is extraordinarily complex, owing to the presence of many components in mixtures. Concentrations of single compounds are very low and plasma concentration of those constituents is often in the range of micro to pictograms per liter, so the analytical techniques to be employed should be sensitive enough to detect the presence of the compounds at that level (Butterweck and Derendorf, 2006; Leucuta and Vlase, 2006; Mukherjee, *et al* 2009). Any rationale to the herbal medicine is likely to be based on the activity of many plant chemical constituents.

Taken together although the study of herbal pharmacokinetics appears to be difficult, the information derived from such investigators will become an important issue to link data from pharmacological assays and clinical effects. In particular, a better understanding of the pharmacokinetics and bioavailability of natural products can help in designing rational dosage regimen and it can help to predict potential botanical product - drug interactions. In addition, those studies would provide supporting evidence for the synergistic nature of herbal medicines and would further help in optimizing the bioavailability and hence the efficacy of herbal products (Gupta, 2010; Modi, 2000).

Pharmacokinetic is therefore a study of how a drug is absorbed, distributed in the organism, metabolized and excreted. There are basically five processes involved:

- **Drug absorption:** Before a drug can exert a pharmacological effect in tissues, it has to be taken into the blood stream - usually via mucous surfaces like digestive tract (intestinal absorption). Absorption is the process that involves drug movement from the site of entry into the bloodstream (Jambhekar and Breen, 2009).

- **Drug distribution:** Distribution is the process by which a drug is transported in body fluids from the blood stream to the tissues of the body. A drug can be administered through a variety of routes; intramuscularly, intravenously, subcutaneously, orally, rectally or via ear, eye, nasal passage etc. After administration, a drug will distribute itself into the all of the body's compartments and tissues that is able to. The time it takes for this to occur is called distribution phase and is usually rapid. A drug is said to be distributed into a theoretical volume, called the volume of distribution (Vd). This volume is usually measured in liters and is considered theoretical, because it is based on sampling drug concentrations immediately after dosing, with the assumption that the drug is uniformly distributed throughout the body (Beelen and Lewis, 2004).

- **Drug concentration:** The concentration of drug in biological fluid. The concentration falling in time.

- **Drug metabolism:** Metabolism is the process by which a drug is chemically inactivated (broken down by enzymes) so that it can be excreted from the body. The metabolism of the drug is mainly by the liver (Jambhekar and Breen, 2009).

- **Drug excretion:** Excretion is the process by which a drug is removed from the site of action and eliminated from the body; a process that takes place in the kidneys. Immediately after a dose of drug is administered, the body begins to eliminate by hepatic metabolism, renal excretion or both. The elimination process can sometimes follow first order kinetics, whereby constant proportion of the drug is eliminated from the body during each unit of time. If the drug goes into several compartments and is eliminated from these compartments at different rates, then the pharmacokinetics becomes more complicated (Brocks, 2010; Jambhekar and Breen, 2009).

Bioavailability

Bioavailability refers to the relative amount of drug from an administered dosage form which enters the systemic circulation and the rate at which the drug appears in the systemic circulation.

Pharmacokinetics in herbal medicine

One important issue which should underline much of the study of herbal pharmacokinetics is that herbs are not usually directly introduced into the bloodstream, oral or tropical routes of administration are preferred. This renders the study of bioavailability of paramount importance for active constituents in plants. Conventional drugs intended for oral use are designed to have good bioavailability. In contrast, phytochemical are of natural origin and may exhibit unusual or poor bioavailability, which may be further compounded by the choice of dosage preparation. In the modern drug development concept it is accepted that medicinal plants act at a chemical level in the body so the knowledge of pharmacokinetic is vital (Mukherjee, 2002; Pal and Shukla, 2003).

The following parameters need to be considered for evaluating the herbal pharmacokinetics:

- Information to further access the traditional use of a medicinal plant and better information on which to base rational doses.
- A better interpretation of scientific information, particularly in vitro research or in vivo studies where the active compounds are administered by injection.

- A better appreciation of safety and toxicity of a plant and anticipation of potential herb-drug interaction.
- Supporting evidence for the synergistic nature of herbal medicine.
- Ways to optimize the bioavailability and hence efficacy of herbal medicines.
- The pharmaceutical preparation.
- The size of the molecule-very large molecules still have some bioavailability (about 1% or less) which may be due to pinocytosis.
- The fat (lipid) solubility of the molecule – The more fats soluble – the better the bioavailability.
- The water solubility of the molecule or a molecule is both water and fat soluble, it will dissolve in the digestive juices and then cross lipid membranes; otherwise, purely water soluble molecules can be expected to have poor bioavailability. Ionization of the molecule means poor bioavailability.
- Specific factors related to crossing the gut wall, e.g. active transport.
- Factors within the gut – interaction with food, stability in the gut, gastric emptying.
- Metabolism in the gut and first – pass metabolism by the liver.
- Individual factors in the patient, including the influence of pathological factors.
- The presence or absence of food may also influence the absorption and bioavailability of plant constituents.

30.1 Sample preparation

A biological sample containing phytoconstituents required sample pretreatment. The sample preparation involves several factors including the nature of the sample blood, urine, etc; the condition of the sample and the concentration level of drug. Selective detection of the drug relative to the matrix is achieved by selecting the suitable stationary phase or even the composition of the mobile phase can be altered to achieve such separation. It is necessary to isolate the phytoconstituents from interfering matrix substances, from protein binding sites and to concentrate the phytoconstituents. Extensive binding of phytoconstituents occurs in the blood. Interference from the matrix components is maximum when the concentration of the drug is very low and special sample preparation is often required. Conditions of the sample also affect such assay, for example, recoveries from fresh blood and the haemolysed one may not be similar by the sample preparation method (Rajani and Kanaki, 2008).

30.2 Optimization of solvent for extraction

Very often there is a need for extracting and then concentrating the analyte solution because of the limitations of the bioanalytical methods while determining the traces especially in the biomedical, forensic and environmental samples. Solvent extraction is the most popular and convenient approach as several parameters can be modified to optimize the extent of extraction. Such modifications include changing the polarity of the organic solvent, changing the pH, ionic nature and strength of the aqueous phase and use of ion pairing agents. The choice of suitable solvent plays major role in achieving maximum percent recovery of the drug from the matrix. Solvent chosen for extraction must provide maximum extraction efficiency with minimum carryover of contaminants. The solvent selected must be of the highest purity and must not be toxic or highly inflammable. It must have suitable volatility to permit evaporation and concentration of the analyte. After extracting the sample by a suitable solvent the analyte solution is further concentrated by evaporation (Jones and Kinghorn, 2006).

30.3 Liquid-Liquid extraction

This technique is based on the separation of analytes from interferences by partitioning the analyte in between two immiscible liquids or phases. Here one phase is matrix and another is organic solvent or mixture of solvents. This type of extraction is achieved by manual shaking or by using commercially available shakers. The analytes extracted into the organic phase are easily recovered by evaporation of the organic solvent under stream of nitrogen or air at low temperatures. Then the residue is reconstituted in mobile phase (usually 0.2 to 1.0 cm^3) and injected. This method is more selective, sensitive and economical. Acceptable percentage recovery is achievable. Convenience ease of use and ready availability of highly purified organic solvent has contributed to its highly wide spread use.

30.4 Statistical method for Bioavailability studies

After a bioavailability study is conducted and the appropriate parameters are determined, the pharmacokinetic data is examined according to a set of predetermined criteria. The statistical methods to be used in bioavailability studies are chosen with careful attention being given to the effect of the variations among individuals and batches of nominally identical manufactured drug products. The planning, analysis

and interpretations of these experiments are not routine problems, but rather require considerable care, consonant with the purpose for which data are to be used.

30.5 Win Nonlin software for pharmacokinetic analysis

Win Nonlin is distributed in two versions, Win Nonlin Standard and Win Nonlin Professional (Win Nonlin, 1999). The standard version of Win Nonlin has been developed for use by scientists involved with nonlinear modeling. It includes features which make it particularly suitable for pharmacokinetic / pharmacodynamic (PK/PD) modeling and noncompartmental analysis. Win Nonlin Professional is particularly suitable for evaluating data from bioavailability and clinical pharmacology studies. Win Nonlin Professional can be used to perform virtually all the analyses and generate all the figures, tables and listing required for regulatory submissions. Win Nonlin functions have been described in the chapter of Materials and Methods. In the current study Win Nonlin was used to generate the requisite pharmacokinetic data like C_{max}, $AUC_{(0-t)}$, $AUC_{(0-\infty)}$, T_{max}, T_{half} and K_{el}. It was also used to generate the fraction of the drug absorbed using the Wagner-Nelson model.

In the present work, pharmacokinetic study of *Mimosa pudica* and *Symplocos racemosa* was conducted on rats. The pharmacokinetic study was carried out to follow the absorption and elimination of marker in a polyherbal combination of *Mimosa pudica* and *Symplocos racemosa* using rat's plasma by HPLC method. The main objective of this study was to investigate possibilities of using any plasma component (a marker), detected by HPLC for following the herbal preparation in the biological system.

30.6 Gallic acid: A therapeutically active constituent

Medicinal plants contain a large number of phytoconstituents belonging to several chemical classes. These phytoconstituents provide definite physiological action to the human body. The presence of phytoconstituents contributes medicinal as well as physiological properties to the plant in the treatment of different ailments. Several studies have reported the presence of phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids, and alkaloids. The phenolic compounds possess biological properties such as antioxidants, antiapoptosis, antiaging, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation

activities (Han *et al*, 2007). Saponins are known to possess anti-inflammatory properties and the property of precipitating and coagulating red blood cells (Yadav and Agarwala, 2011). Steroids have been reported to have antibacterial properties (Raquel, 2007). Alkaloids are associated with the analgesic, antispasmodic and antibacterial activity (Stray, 1998; Yadav and Agarwala, 2011). Glycosides are known lower blood pressure (Yadav and Agarwala, 2011).

Gallic acid is a type of phenolic acid which is known to have anti-inflammatory, anti-mutagenic, anti-cancer, anti-oxidant and anti-diabetic activities (Borde *et al*, 2011). The anti-diabetic agents like metformin, thiazolidinediones have been widely used in the management of hyperandrogenism associated with insulin resistance in PCOS patients (Homburg, 2008). Since Gallic acid from plants has anti-diabetic properties, it can be evaluated for its therapeutic efficacy in the treatment of PCOS. Therefore Gallic acid is selected as a marker in the present research work and its bioavailability is determined with the help of a pharmacokinetic parameters. The earlier studies have reported the presence of Gallic acid in *Mimosa pudica* and *Symplocos racemosa* (Azmi *et al*, 2011; Borde *et al*, 2011; Nataraj *et al*, 2012; Saraswat and Pokharkhar, 2012).

A combination of ethanolic extract of *Mimosa pudica* (Whole plant powder) and *Symplocos racemosa* (Stem bark powder) in 1:1proportion was used as test material in this study.

It is carried out in the following stages:

- Detection of a distinctive peak in rat plasma spiked with polyherbal combination.
- Identifying and establishing a marker using plasma from a rat administered with polyherbal combination.
- Establishing the linearity of the marker peak using spiked rat plasma.
- Determination of bioavailability with the help of pharmacokinetic parameters for the marker using *in vivo* study on rat administered with polyherbal combination.

31. Detection of distinctive peak in rat plasma spiked with polyherbal combination (*in vitro* study)

In vitro study is essential to establish and standardize experimental conditions which can be used for the *in vivo* study.

31.1 Animal model

Animals used for the investigation are Albino Wistar rats, procured from Bharat Serums Pvt Ltd., Mumbai, India. Weights of the animals ranged between 180 to 220g.

31.2 Blood Collection

The retro orbital from albino Wistar rats was used for blood collection. It was carefully transferred to a sterile, heparinized micro centrifuge 1.5 cm^3 Eppendorf tubes ensuring that there was no haemolysis and centrifuged at 2000 rpm for 10 minutes. Plasma was separated and subjected to further analysis.

31.3 Preparation of Gallic acid standard

10 mg of Gallic acid pure standard was weighed in 10 mL of standard volumetric flask and dissolved in 10 mL of methanol to get a stock solution of a concentration of 1000 ppm. From this stock further, serial dilution with mobile phase was given to get following concentration of Gallic acid 100 ppm, 10 ppm and 1 ppm. The solutions were protected from light and were prepared fresh. 20 µl of Gallic acid standard 10 ppm and 1 ppm were injected into the HPLC system.

31.4 Preparation of plasma samples and extraction procedure

0.5 cm^3 of plasma was taken in clean and dry tubes. 5 cm^3 of ethyl acetate was added in each test tube and the tubes were shaken for 10 mins on rotary shaker. The tubes were centrifuged for 10 mins at 4000 rpm. 4 mL of organic layer was transferred to evaporating tubes and the organic layer was evaporated at 40 º C under nitrogen stream in low volume evaporator. The residue was then reconstituted with 200 µl of mobile phase and 20 µl was injected into the HPLC system. Since Gallic acid is light sensitive so all the processing was carried out sodium vapour lamp environment.

The choice of suitable solvent plays a major role in achieving maximum percent recovery of the drug from matrix. Thus, the entire range of solvents from polar to non polar, which are immiscible in plasma like n-hexane, dichloromethane, chloroform, ethyl acetate are tried. The extraction was found to be maximum in ethyl acetate as compared to other solvents and therefore it was selected as the most suitable solvent for extraction.

31.5 Chromatographic conditions

Pump: Jasco HPLC-PU 980 pump

Detector: Jasco, MD 910 multi wavelength (PDA) detector

Column: Cosmosil C_{18}, 150 mm × 4.6 mm i.d., 5µ

Injection volume: 20 µl

Flow rate: 1 cm^3/min

Wavelength: 215 nm for Gallic acid

Mobile phase : Water : Acetonitrile (95:5 v/v) pH 3.0

Ethyl acetate extracts of both, Blank plasma, plasma spiked with Gallic acid (10 ppm) are injected into the HPLC system. Gallic acid (marker) peak was identified after comparing with the blank plasma pattern and was further applied for *in vivo* pilot pharmacokinetic study.

31.6 Observation

When the chromatograms of Gallic acid standard was compared with the chromatogram of ethyl acetate extract of blank plasma and the chromatogram of ethyl acetate extract of plasma spiked with polyherbal combination and plasma spiked with Gallic acid (1 ppm) a single marker peak was observed in UV mode at 215 (Figure 92, Figure 93, Figure 94, Figure 95). This marker peak was not observed in the blank plasma.

Figure 92: Representative chromatogram of Gallic acid standard 1ppm

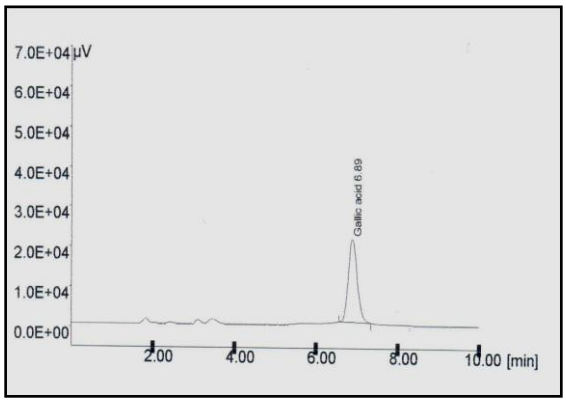


Figure 93: Representative chromatogram of Blank rat plasma

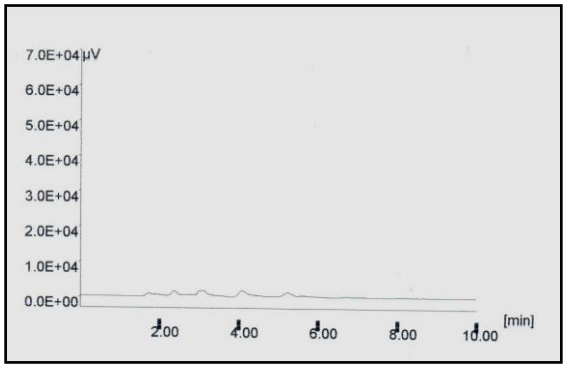


Figure 94: Representative chromatogram of Gallic acid (1ppm) from polyherbal combination spiked in rat plasma

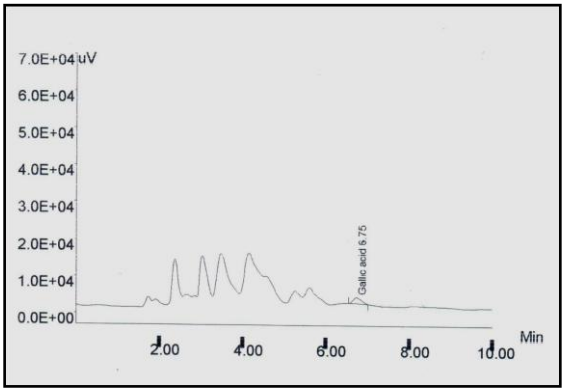


Figure 95: Representative chromatogram of Gallic acid (10 ppm) spiked in rat plasma

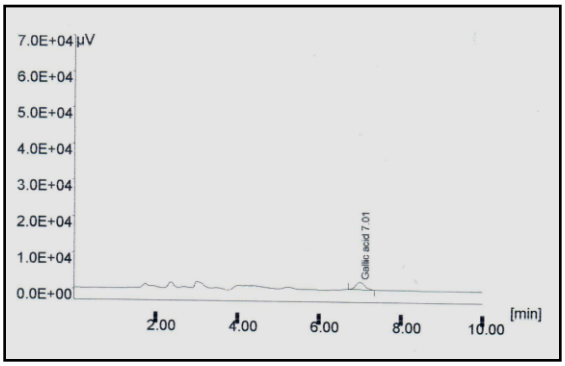


Table 39: Table of Distinctive Peak for Spiked plasma obtained at RT of Gallic acid

Peak no.	Retention (Rt) time in minutes (Blank plasma)	Retention (Rt) time in minutes (Gallic acid standard 1 ppm)	Retention (Rt) time in minutes (plasma spiked with polyherbal combination)	Retention (Rt) time in minutes (plasma spiked with Gallic acid 1 ppm)
1	-	6.89	6.75	7.01

The reproducibility of the results of this study provided a rationale for continuing to use the same methodology for *in vivo* studies carried out in rats.

32. Identifying and establishing Gallic acid marker using plasma from rat administered with the polyherbal combination

A preliminary study was carried out to establish the marker using plasma from a single rat which was administered with the polyherbal combination.

32.1 Materials and methods

Healthy Albino Wistar rats weighing between 180-220 g were used in this study. Oral route of administration was selected for the present study. Rats were kept fasting 18 hours prior to the administration of polyherbal combination. Blank sample (zero hour) of plasma was collected from the rats and was fed orally (using gavage number 16) with 1g/kg body weight of polyherbal combination prepared in 10 mL of distilled water. Blood samples were collected at 30, 60, 90, 120, 180 and 240 minutes after the oral dose. 3 mL of blood was collected in heparinized eppendorf tubs at each sampling point. The plasma was separated and subjected to further analysis.

32.2 Observations

Comparison of rat plasma after 1.50 hr of administration of polyherbal combination with blank plasma indicated the peak of retention time 6.88 to be very distinctive (Figure 5 and Figure 6). This was considered the possible Gallic acid (marker) peak. The peak is detectable both in the fingerprint of polyherbal combination, Gallic acid standard (1 ppm) and the rat plasma spiked with Gallic acid (10 ppm) (Figure 92, Figure 94 and Figure 95). The linear response of Gallic acid (marker) peak was used for evaluation.

Figure 96: Representative chromatogram of 0.00 hr. Rat plasma sample

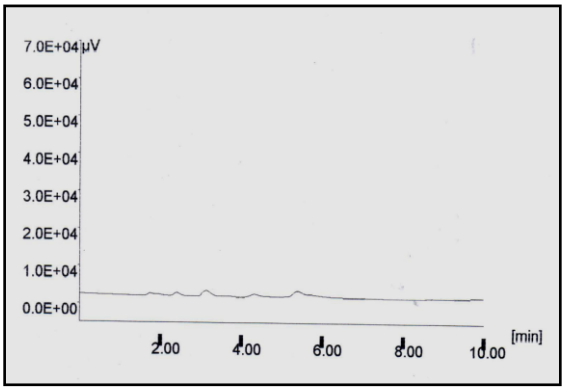
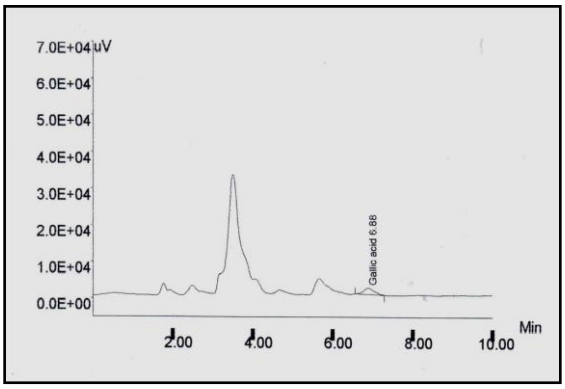


Figure 97: Representative chromatogram of 1.50 hr. post dose Rat plasma sample



33. Establishing the linearity of the Gallic acid (marker) peak using rat plasma spiked with Gallic acid.

A study was carried out to demonstrate the linearity of the marker peak by using rat plasma spiked with varying concentration of Gallic acid. For this a stock solution (1000 ppm) of Gallic acid standard was prepared. The stock was serially diluted to obtain working solutions viz. 100 ppm, 10 ppm and 1 ppm.

From these stock solutions specified volumes were spiked separately into 0.5 mL of plasma each to obtain 40.00 ng/ mL, 80.00 ng/ mL, 120.00 ng/ mL, 200.00 ng/ mL, 400.00 ng/ mL, 600.00 ng/ mL and 800.00 ng/ mL. The response was found to be linear between 40.00 ng/ mL and 800.00 ng/ mL.

Sample processing was same as in the prior study by liquid-liquid extraction procedure.

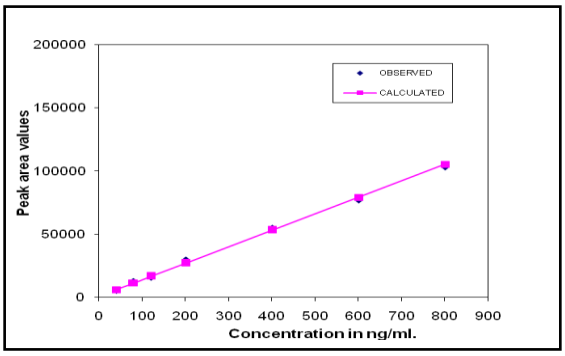
Table 40: Linearity of Gallic acid

Concentration (ng/mL)	Area (215 nm)	Calculated concentration (ng/mL)	% Nominal
40.00	5526	34.67	86.68
80.00	12273	86.45	108.07
120.00	16024	115.24	96.03
200.00	29713	220.30	110.15

400.00	55066	414.88	103.72
600.00	76983	583.08	97.18
800.00	103342	785.38	98.17
RSQ	**0.9981**	**% Nominal = Calculated concentration / Actual concentration × 100**	
Slope (m)	**130.30**		
Intercept (c)	**1008.33**		

(y = mx + c; where y = peak area; m = slope; x = concentration; c = intercept)

Figure 98- Plasma Linearity of Gallic acid by (Regression curve)



The above linearity was used as the basis for calculating the relative concentration of the marker in plasma during *in vivo* study of polyherbal combination.

Figure 99: Representative chromatogram of Blank Rat plasma

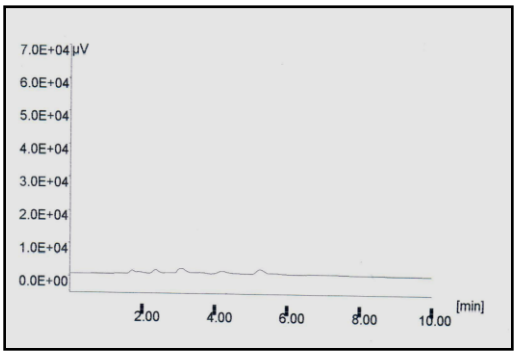


Figure 100: Representative chromatogram of Gallic acid (40.00 ng/mL) spiked in rat plasma

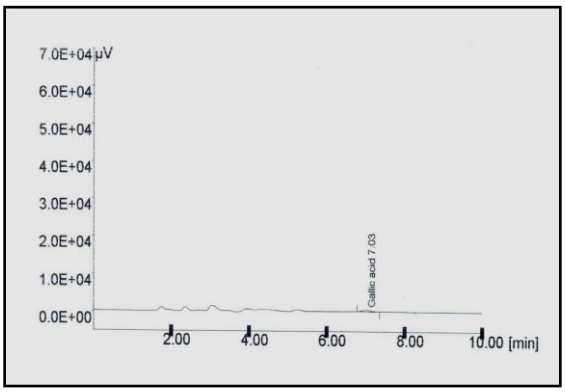
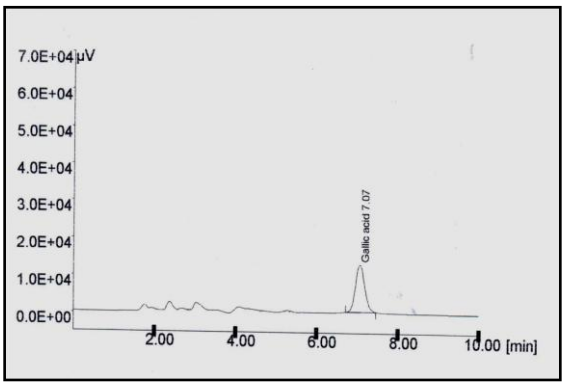


Figure 101: Representative chromatogram of Gallic acid (800.00 ng/mL) spiked in rat plasma



34. Determination of bioavailability with the help of pharmacokinetic parameters for the marker using *in vivo* study on rat administered with polyherbal combination

34.1 Materials and methods

Healthy albino wistar rats weighing between 180 to 220 g were used in this study. Oral route of administration was selected for the present study. Rats were kept fasting 18 hours prior to the administration of polyherbal combination. Blank sample (zero hour) of plasma was collected from the rat and was fed orally (using gavage number 16) with 1g/kg body weight of polyherbal combination prepared in 10 mL of distilled water. Blood samples were collected at 0.50, 1.00, 1.50, 2.00, 3.00, 4.00, 6.00, 12.00 and 24.00 hours post dose. 3 mL of blood was collected in heparinized eppendorf tubs at each sampling point. The plasma was separated and subjected to further analysis.

34.2 Extraction from plasma

0.5 cm^3 of plasma was taken in clean and dry tubes. 5 cm^3 of ethyl acetate was added in each test tube and the tubes were shaken for 10 mins on rotary shaker. The tubes were centrifuged for 10 mins at 4000 rpm. 4 mL of organic layer was transferred to evaporating tubes and the organic layer was evaporated at 40 º C under nitrogen stream in low volume evaporator. The residue was then reconstituted with 200 µl of mobile phase and 20 µl was injected into the HPLC system with the same chromatographic conditions as explained in *in vitro* study.

34.3 Observations

The marker peak of Gallic acid was not observed at 0.00 hrs (Figure 96); however the marker peak in plasma increases gradually and reaches its maximum at 1.50 hr (Figure 97). The peak height in plasma decreases gradually thereafter. At 24 hrs, the peak height was found to be least indicating elimination.

The pharmacokinetic study of polyherbal combination thus showed a typical absorption – elimination pattern as reflected by the peak area of the marker peak for Gallic acid in the plasma after oral administration in the rats (Table 41).

Table 41: Pharmacokinetics of the Gallic acid (marker) with respect to the peak areas

Sampling hours	Concentration (Concentration expressed as relative concentration of the Gallic acid in ng/mL (Mean ± SE) n=3
0.00	0.00
0.50	53.56 ± 6.79
1.00	99.23 ± 13.00
1.50	160.51 ± 9.72
2.00	120.49 ± 8.04
3.00	74.93 ± 9.34
4.00	41.30 ± 8.05
6.00	19.98 ± 5.51
12.00	6.01 ± 2.15
24.00	1.53 ± 0.98

Figure 102: Representative chromatogram of 0.00 hr. rat plasma sample

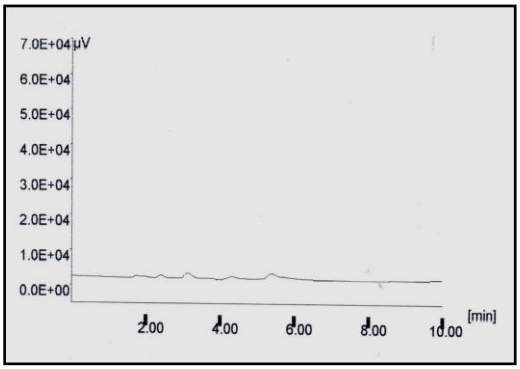
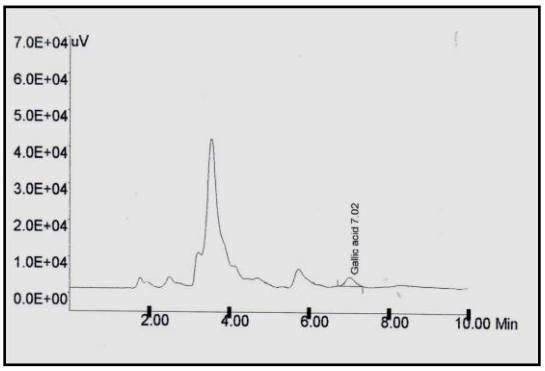


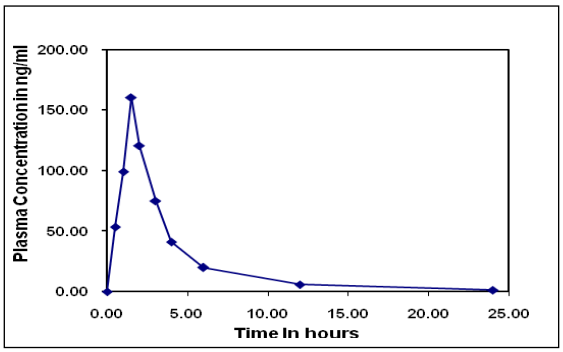
Figure 103: Representative chromatogram of 1.50 hr. post dose rat plasma sample



35. Results

Plasma concentration of the marker Gallic acid from polyherbal combination was determined using HPLC. A graph showing the absorption – elimination pattern of the marker from polyherbal combination is given in figure 12 as a plot of marker peak area against time. The pharmacokinetics parameters generated for polyherbal combination after oral administration using absorption – elimination curve is shown in Figure 104.

Figure 104: Absorption – Elimination curve for the marker in rat plasma (Mean plasma concentration vs Time curve)



Using the linearity obtained for plasma spiked with Gallic acid during *in vitro* study as a reference (Table 40), the concentration of the marker in plasma during *in vivo* study of polyherbal combination, as a relative concentration of Gallic acid was calculated using Win Nonlin as shown in Table 42.

Table 42: Pharmacokinetic parameters obtained from Win Nonlin analysis

Parameters	Results (Mean ± SE) n = 3
C_{max}	160.52 ± 16.83 ng/mL
T_{max}	1.50 hrs
$AUC_{(0-t)}$	498.99 ± 135.22 ng/mL*hr
$AUC_{(0-\infty)}$	515.09 ± 141.43 ng/mL*hr
$T_{1/2-\lambda-z}$	3.74 ± 1.96 hr
K_{el}	0.22 ± 0.10 hr^{-1}

Table 43: Blood concentrations and associated data for Gallic acid for current study (Dosing is oral)

Time in hours	Concentration C_P in ng/mL	tn [AUC] tn-1	[AUC] 0-T	k [AUC] 0-T	C_P + k [AUC] 0-T	Ab/Ab^{∞}	1- Ab/Ab^{∞}	% drug Absorbed In vivo
0.00	0.00	0.00	0	0.00	0.00	0.00		0.00
0.50	53.56	13.39	13.39	1.87	55.44	0.77	0.23	76.88
1.00	99.23	38.20	51.59	7.22	106.45	1.48	-0.48	147.62
1.50	160.51	64.94	116.52	16.31	176.83	2.45	-1.45	245.21
2.00	120.49	70.25	186.29	26.08	146.57	2.03	-1.03	203.25
3.00	74.93	97.71	282.21	39.51	114.43	1.59	-0.59	158.69
4.00	41.30	58.11	338.66	47.41	88.71	1.23	-0.23	123.02
6.00	19.98	61.28	397.39	55.63	75.61	1.05	-0.05	104.85
12.00	6.01	77.96	467.16	65.40	71.41	0.99	0.01	99.03
24.00	1.53	45.21	506.45	70.90	72.43	1.00	0.00	100.44

Drug concentrations in blood at various time intervals are listed in Table 43.

Assuming that the drug follows a one compartment model, the percent drug absorbed versus time is determined by Wagner – Nelson method (Shargel, 1999).

The AUC (Area under curve) is approximated by the trapezoidal rule. This method is fairly accurate when there are sufficient data points. The area between each time point is calculated as follows:

$$[AUC]_{t_{n-1}}^{t_n} = \frac{C_{n-1} + C_n}{2}(t_n - t_{n-1})$$

Where C_n and C_{n-1} are concentrations and t_n and t_{n-1} are time points.

To obtain $[AUC]_0^\infty$ all the area portions under the curve from zero to infinity are added.

Figure 105: Mean plasma concentration vs Time curve for marker Gallic acid from Wagner Nelson method

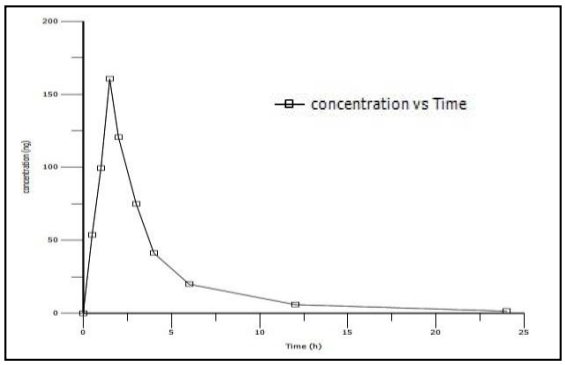


Figure 106: AUC vs Time for marker Gallic acid from Wagner – Nelson method

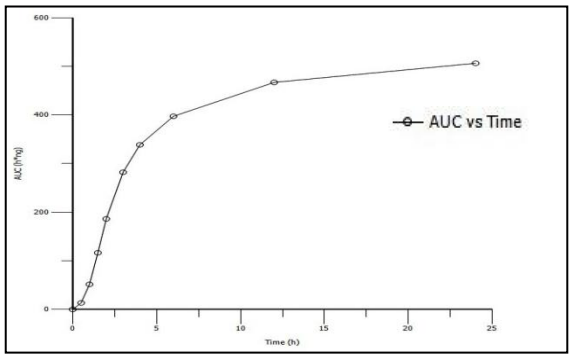
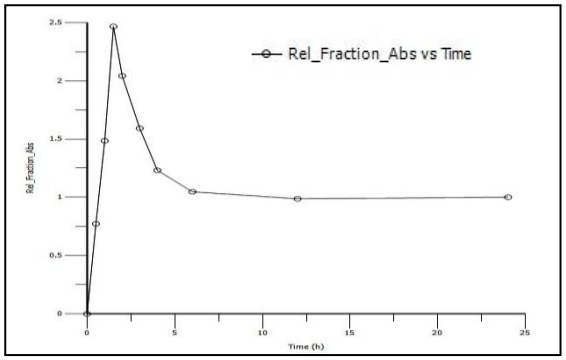


Figure 107: Relative fraction of marker Gallic acid absorbed vs Time from Wagner – Nelson method



In present bioavailability study, 24 hours shows minimum blood concentration. By applying Wagner – Nelson method it can be deduced that over 24 hr period 100.44% of Gallic acid is absorbed indicating 100% bioavailability when administered in rats in the form of polyherbal combination. As the drug approaches 100% absorption, C_p

becomes very small and difficult assay accurately. It will be of great therapeutic interest to evaluate relative bioavailability of Gallic acid from polyherbal combination in humans.

Clinical signs:

The treated animals did not show any abnormal clinical signs throughout the study

Animal no.	Sex	Time after treatment	
		1 hr	24 hr
1	female	NAD	NAD
2	Female	NAD	NAD
3	female	NAD	NAD

NAD – No abnormality detected

36. Conclusion

Marker, Gallic acid of polyherbal combination showed a typical absorption, elimination pattern. The study demonstrates the feasibility of developing methods to detect markers of medicinal plants in biological matrix using similar approach. Gallic acid possesses many potential therapeutic properties including anti-cancer, anti-oxidant and anti-diabetic properties. All these properties make Gallic acid the pharmacologically important compound. The marker, Gallic acid is not claimed to be the main active principle but its absorption-elimination pattern may be considered to be the relative absorption – elimination pattern for the polyherbal combination. It can be used to fix the therapeutic window for the polyherbal preparation. The pharmacokinetic parameters for Gallic acid obtained from Win Nonlin analysis are C_{max} 160.52 ± 16.83 ng/mL, T_{max} 1.50 hrs, $AUC_{(0-t)}$ 498.99 ± 135.22 ng/mL*hr, $AUC_{(0-\infty)}$ 515.09 ± 141.43 ng/mL*hr, $T_{1/2}$ 3.74 ± 1.96 hr and K_{el} 0.22 ± 0.10 hr^{-1}. The percentage of Gallic acid absorbed over 24 hr period is found to be 100.44%.

Thus such a pharmacokinetic study in animal model can be safely used to test formulations for their bioavailability by comparing peak plasma concentration of such markers especially to compare equivalence between formulations from different manufactures, different batches etc.

37. References

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Details of Junior Research Fellow

S. P Mandali's
Ramnarain Ruia College
NAAC Reaccreditation: 'A' Grade 3.65 CGPA Estd. 1937

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Annexure - VI

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

PROFORMA FOR SUPPLYING THE INFORMATION IN RESPECT OF THE STAFF APPOINTED UNDER THE SCHEME OF MAJOR RESEARCH PROJECT

UGC FILE No.: MRP-MAJOR-BOTA-2013-22123 (F. NO. -43-121/2014(SR)

YEAR OF COMMENCEMENT: 18th September 2015

TITLE OF THE PROJECT: Evidence Based Evaluation of Medicinal Plants Used for Treating Polycystic Ovary Syndrome

1	Name of the Principal Investigator	Dr. Sunita Shailajan			
2	Name of the University/ College	Ramnarain Ruia College, Matunga, Mumbai – 19			
3	Name of the Research Personnel appointed	Ms. Sahina Bhosale			
4	Academic qualification:	Qualification	Year	Marks	Percentage
		M.Sc	2015	429/600	71.50%
		PhD (Pursuing)	2017	–	–
5	Date of joining	18th September, 2015			
6	Date of birth of research personnel	30th April, 1993			
7	Amount of HRA, if withdrawn	Not applicable			
8	Number of candidates applied for the post	04			

CERTIFICATE

This is to certify that all the rules and regulations of UGC Major Research Project outlined in the guidelines have been followed. Any lapse on the part of the University will liable to terminate of said UGC project.

SIGNATURE OF THE PRINCIPAL INVESTIGATOR

PRINCIPAL INVESTIGATOR

REGISTRAR/PRINCIPAL
(Seal)
Principal
Ramnarain Ruia College,
Matunga, Mumbai-400 019.

SIGNATURE OF THE CO-INVESTIGATOR

Statement of Expenditure from 1st July 2015- 31st March 2016

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Annexure - III

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

STATEMENT OF EXPENDITURE IN RESPECT OF MAJOR RESEARCH PROJECT

1. Name of Principal Investigator : Dr. Sunita Shailajan
2. Deptt. of Principal Investigator : Department of Botany
University/College : *S.P.Mandali's* Ramnarain Ruia College, Matunga (E), Mumbai- 400 019
3. UGC approval Letter No. and Date: F. NO. - 43-121/2014(SR)
4. Title of the Research Project : Evidence Based Evaluation Of Medicinal Plants Used For Treating Polycystic Ovary Syndrome
5. Effective date of starting the project 1st July 2015
6. a. Period of Expenditure : From 1st July 2015 to 31st March 2016
   b. Details of Expenditure : Rs. 7,08,422/-

S.No.	Item	Amount Approved(Rs.)	Expenditure Incurred(Rs.)
i.	Books & Journals	30000.00	15107.00
ii.	Equipment	400000.00	400000.00
iii.	Contingency	45000.00	16474.00
iv.	Field Work/Travel (Give details in the proforma at Annexure-IV).	15000.00	8049.00
v.	Hiring Services	50000.00	18645.00
vi.	Chemicals & Glassware	100000.00	75080.00
vii.	Overhead	99000.00	99000.00
viii.	Any other items (Please specify) Project fellowship	300000.00	76067.00
	Total	**1039000.00**	**708422.00**

c . Staff

Date of Appointment: 18th September 2015

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S.No	Items	From	To	Amount Approved (Rs.)	Expenditure incurred (Rs.)
1.	Honorarium to PI (Retired Teachers) @ Rs. 18,000/-p.m.	--	--	--	--
2.	**Project fellow:** **NET/GATE qualified**-Rs. 16,000/- p.m.for initial 2 years and Rs. 18,000/- p.m. for the third year. **Non-GATE/Non-NET**- Rs.14,000/- p.m. for initial 2 years and Rs. 16,000/- p.m. for the third year.	18th September 2015	31st March 2016	300000.00	76067.00

1. It is certified that the appointment(s) have been made in accordance with the terms and conditions laid down by the Commission.
2. If as a result of check or audit objection some irregularly is noticed at later date, action will be taken to refund, adjust or regularize the objected amounts.
3. Payment @ revised rates shall be made with arrears on the availability of additional funds.
4. It is certified that the grant of Rs. 10,39,000.00 (Rupees Ten Lakhs Thirty Nine Thousand Only) received from the University Grants Commission under the scheme of support for Major Research Project entitled 'Evidence Based Evaluation Of Medicinal Plants Used For Treating Polycystic Ovary Syndrome' vide UGC letter No. F. No. - 43-121/2014 (SR) dated 12th August 2015 out of which Rs. 7,08,422.00 (Rupees Seven Lakhs Eight Thousand Four Hundred and Twenty Two Only) has been fully utilized for the purpose for which it was sanctioned and in accordance with the terms and conditions laid down by the University Grants Commission.

SIGNATURE OF THE PRINCIPAL INVESTIGATOR
PRINCIPAL INVESTIGATOR

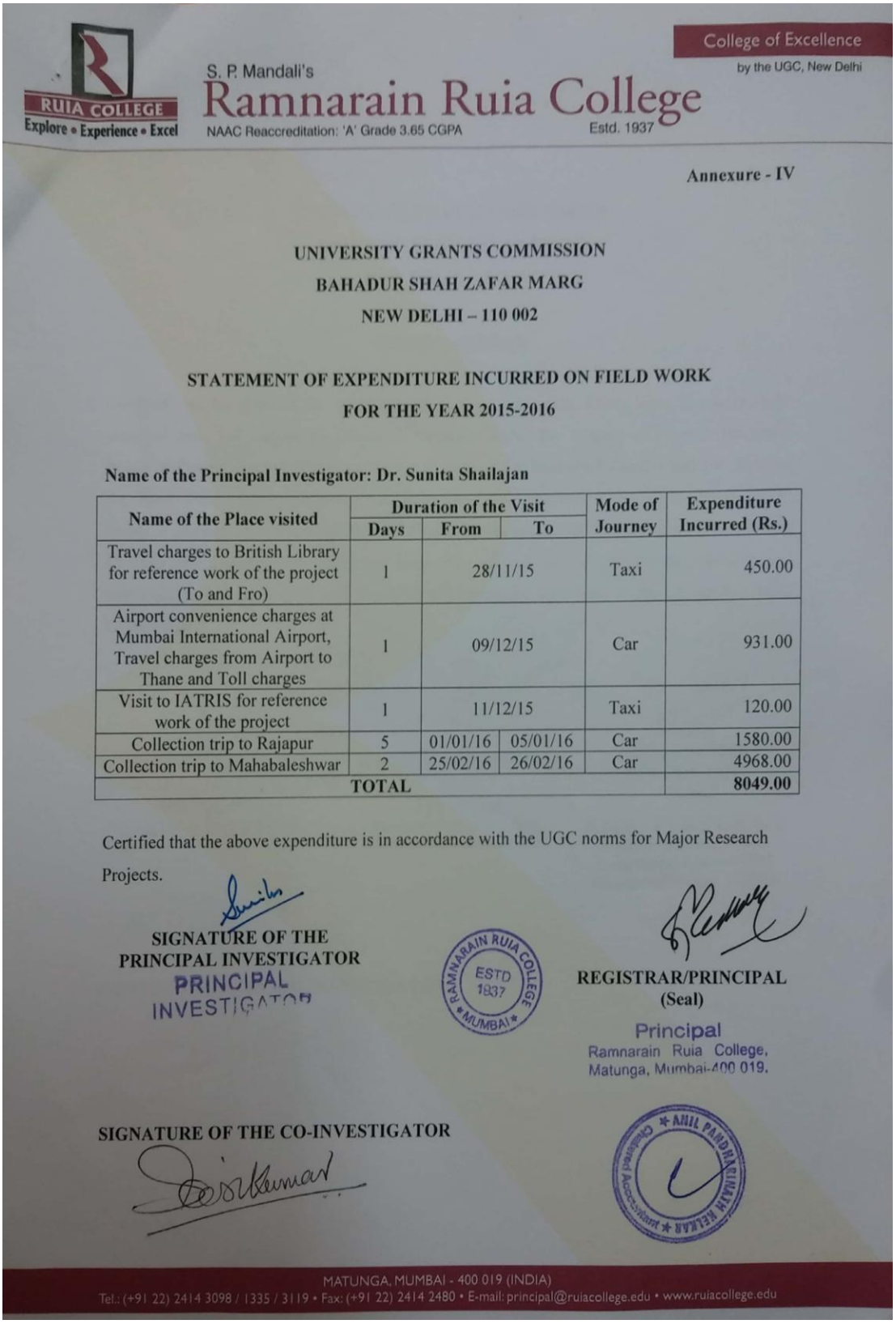
RAMNARAIN RUIA COLLEGE ESTD 1937 MUMBAI

REGISTRAR/PRINCIPAL
(Seal)
Principal
Ramnarain Ruia College,
Matunga, Mumbai-400 019.

SIGNATURE OF THE CO-INVESTIGATOR

ANIL PADMANABHAN

Statement of Expenditure incurred on Field work for the year 2015-2016



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Annexure - IV

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

STATEMENT OF EXPENDITURE INCURRED ON FIELD WORK
FOR THE YEAR 2015-2016

Name of the Principal Investigator: Dr. Sunita Shailajan

Name of the Place visited	Duration of the Visit			Mode of Journey	Expenditure Incurred (Rs.)
	Days	From	To		
Travel charges to British Library for reference work of the project (To and Fro)	1	28/11/15		Taxi	450.00
Airport convenience charges at Mumbai International Airport, Travel charges from Airport to Thane and Toll charges	1	09/12/15		Car	931.00
Visit to IATRIS for reference work of the project	1	11/12/15		Taxi	120.00
Collection trip to Rajapur	5	01/01/16	05/01/16	Car	1580.00
Collection trip to Mahabaleshwar	2	25/02/16	26/02/16	Car	4968.00
TOTAL					8049.00

Certified that the above expenditure is in accordance with the UGC norms for Major Research Projects.

SIGNATURE OF THE
PRINCIPAL INVESTIGATOR
PRINCIPAL
INVESTIGATOR

RAMNARAIN RUIA COLLEGE ESTD 1937 MUMBAI

REGISTRAR/PRINCIPAL
(Seal)
Principal
Ramnarain Ruia College,
Matunga, Mumbai-400 019.

SIGNATURE OF THE CO-INVESTIGATOR

ANIL PANDURANG KELKAR Chartered Accountant

MATUNGA, MUMBAI - 400 019 (INDIA)
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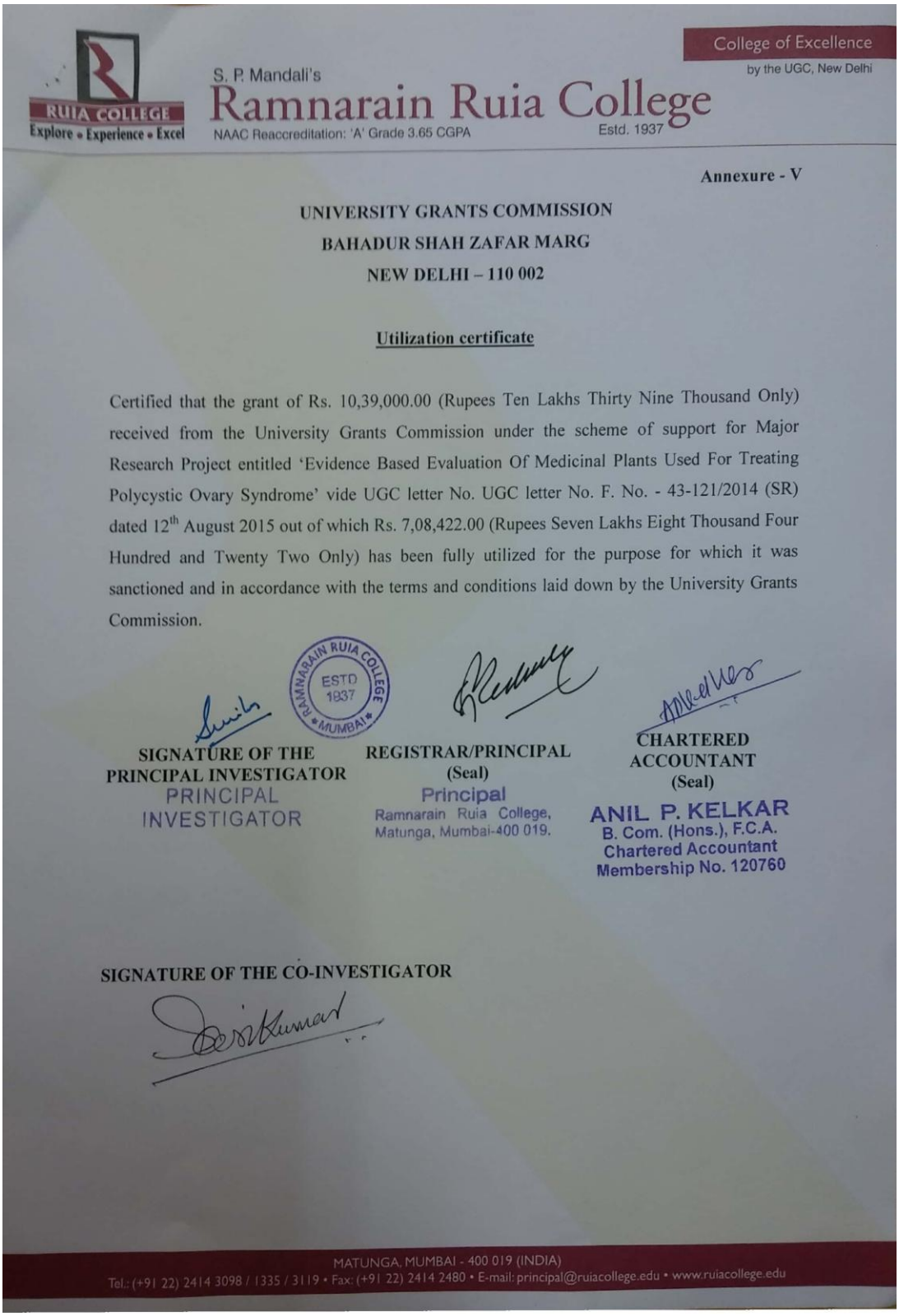
Utilization Certificate from 1st July 2015- 31st March 2016



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Annexure - V

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

Utilization certificate

Certified that the grant of Rs. 10,39,000.00 (Rupees Ten Lakhs Thirty Nine Thousand Only) received from the University Grants Commission under the scheme of support for Major Research Project entitled 'Evidence Based Evaluation Of Medicinal Plants Used For Treating Polycystic Ovary Syndrome' vide UGC letter No. UGC letter No. F. No. - 43-121/2014 (SR) dated 12th August 2015 out of which Rs. 7,08,422.00 (Rupees Seven Lakhs Eight Thousand Four Hundred and Twenty Two Only) has been fully utilized for the purpose for which it was sanctioned and in accordance with the terms and conditions laid down by the University Grants Commission.

RAMNARAIN RUIA COLLEGE ESTD 1937 MUMBAI

SIGNATURE OF THE PRINCIPAL INVESTIGATOR
PRINCIPAL
INVESTIGATOR

REGISTRAR/PRINCIPAL
(Seal)
Principal
Ramnarain Ruia College,
Matunga, Mumbai-400 019.

CHARTERED ACCOUNTANT
(Seal)
ANIL P. KELKAR
B. Com. (Hons.), F.C.A.
Chartered Accountant
Membership No. 120760

SIGNATURE OF THE CO-INVESTIGATOR

MATUNGA, MUMBAI - 400 019 (INDIA)
Tel.: (+91 22) 2414 3098 / 1335 / 3119 • Fax: (+91 22) 2414 2480 • E-mail: principal@ruiacollege.edu • www.ruiacollege.edu

Detailed Statement of Expenditure 1st July 2015- 31st March 2016

Ramnarain Ruia College, Matunga Mumbai

Name of the Scheme: University Grants Commission (Major Research Project)

Sanctioning Authority: (F.No. -43-121/2014(SR) dated 12th August 2015

Sr.no	Date	Code	Particulars	Non - recurring		Recurring						Total Expenditure
				Books & Journals	Equipments	Contingency	Travel & field work	Hiring services	Chemicals & Consumables	Institutional overhead	Project Fellowship	Total
1	7/7/2015	D0382	Cheque paid to Academic book house	8502								8502
2	16/10/2015	C1457	Honorarium: Dr. Savita Kulkarni						500			500
3	16/10/2015	C1458	Honorarium: Dr. Urmi Pakar						500			500
4	16/10/2015	C1459	Honorarium: Dr. Behnaz Patel						500			500
5	16/10/2015	C1460	Honorarium: Dr. Sunita Shalajan						500			500
6	16/10/2015	C1461	Honorarium: Dr. Sasikumar Menon						500			500
7	19/10/2015	B1021	Cheque paid to Gautam Advertisers (Advertisement for the interviews of Junior Research Fellow)			8428						8428
8	19/10/2015	C1594	Payment as per Voucher: Vrindavan (Refreshment charges for the interviewers)						251			251
9	31/10/2015	B1110	Cheque paid to Bhagyesh Enterprise (Purchase of chemicals HPLC grade ACN, MeOH, Ethylacetate)						18049			18049
10	5/11/2015	B1129	Fellowship paid to Subina Bhosale for the month of September and October 2015								19867	19867
11	20/11/2015	C1809	Prof Tax (October 2015)								200	200
12	4/12/2015	B1298	Fellowship paid to Subina Bhosale for the month of November 2015								13800	13800
13	8/12/2015	C1897	Prof Tax (November 2015)								200	200
14	3/12/2015	C1883	Payment as per Voucher: Consumables (Charges for stationary, safety evaluation of Mimosa pudica on rats)			677			5290			5967
15	14/12/2015	C1939	Payment as per Voucher: Miscellaneous (Charges for purchase of plant raw material and plastic bottles for the project)			126			1905			2031
16	14/12/2015	C1940	Payment as per Voucher: Miscellaneous (Charges for stationary, Collection and processing of plant raw material)			2995		400	1774			5169
17	17/12/2015	C1983	Payment as per Voucher: Plant Collection & Travel charges				931	6068	80301			13021
18	18/12/2015	C2003	Payment as per Voucher: Publication charges	6605								6605
19	22/12/2015	B1399	Cheque paid to Arihant enterprises (Purchase of HPLC grade ACN, MeOH, Cyclohexane and TLC sheets)						9215			9215
20	19/01/2016	B1483	Fellowship paid to Subina Bhosale for the month of December 2015								13800	13800
21	15/01/2016	C2178	Prof Tax (December 2015)								200	200
22	19/01/2016	B1484	Cheque paid to Arihant enterprises (Purchase of HPLC grade, ACN and Blotting paper)						2205			2205
23	23/02/2016	C2369	Payment as per Voucher: Consumables (Charges for stationary, purchase of sample for analysis, processing of raw material and travel expense for reference work)			1249	450		3423			5122
24	23/02/2016	C2371	Payment as per Voucher: Plant collection (Charges for payment to locals for Mimosa pudica and the expenses)				1580	11135	2325			15040
25	24/02/2016	B1568	Fellowship paid to Subina Bhosale for the month of January 2016								13800	13800
26	26/02/2016	C2398	Prof Tax (January 2016)								200	200
27	29/02/2016	B1609	Payment as per Voucher (Overhead charges 10% of approved recurring grant)							99000		99000

28	3/3/2016	B1632	Cheque paid to: Anchrom Lab suppliers (Photodocumentation unit)		400000							400000
29	3/3/2016	B1635	Fellowship paid to Sahina Bhosale for the month of February 2016								13700	13700
30	11/3/2016	C2507	Prof Tax (February 2016)								300	300
31	3/3/2016	C2431	Payment as per Voucher: Miscellaneous (Charges for stationary, purchase of sample for analysis, processing of raw material and travel expense for reference work)			1808	120		3131			5059
32	18/3/2016	C2560	Payment as per Voucher: Plant collection (Charges for collection of Symplocos racemosa from Mahabaleshwar, fuel charges, toll charges, refreshment charges and drivers allowance)				4968		7075			12043
33	28/3/2016	B1784	Cheque paid to: Arihant enterprises (Purchase of acetone and pyridine; n-hexane, chloroform, cyclohexane, 1-propanol, formic acid, acetic acid, HCl, DMSO)						6099			6099
34	28/3/2016	C2615	Payment as per Voucher: Miscellaneous (Charges for stationary, refilling of nitrogen gas cylinder, purchase of sample for analysis)			1191		1050	3808			6049
			TOTAL	15107	400000	16474	8049	18645	75080	99000	76067	708422

Certified that the amount of Rs 708422.00 (Rs Seven lakhs eight thousand four hundred and twenty two only) has been spent for the purpose for which the grant was sanctioned and in accordance with the terms and conditions laid down in F. No. - 43-121/2014 (SR) dated 12th August, 2015

Place: Mumbai
Date: 08 AUG 2016

Dr. Sunita Shailajan
Principal Investigator

PRINCIPAL
INVESTIGATOR

RAMNARAIN RUIA COLLEGE ESTD 1937 MUMBAI

Prof (Dr.) Suhas Pednekar
Principal

Principal
Ramnarain Ruia College,
Matunga, Mumbai-400 019.

Chartered Accountant

ANIL P. KELKAR
B. Com. (Hons.), F.C.A.
Chartered Accountant
Membership No. 120760

Statement of Expenditure from 1st April 2016- 1st January 2017

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Annexure - III

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

STATEMENT OF EXPENDITURE IN RESPECT OF MAJOR RESEARCH PROJECT

1. Name of Principal Investigator: Dr. Sunita Shailajan
2. Deptt. of Principal Investigator: Department of Botany
University/College: *S.P.Mandali's* Ramnarain Ruia College, Matunga (E), Mumbai- 400 019
3. UGC approval Letter No. and Date: F. NO. - 43-121/2014(SR)
4. Title of the Research Project: Evidence Based Evaluation Of Medicinal Plants Used For Treating Polycystic Ovary Syndrome
5. Effective date of starting the project: 1st July 2015
6. a. Period of Expenditure: From 1st July 2015 to 1st January 2017
   b. Details of Expenditure: Rs. 9,31,432/-

S.No.	Item	Amount Approved (Rs.)	Expenditure Incurred (1st July 2015 to 31st March 2016) (Rs.)	Expenditure Incurred (1st April 2016 to 1st January 2017) (Rs.)	Total Expenditure (Rs.)
i.	Books & Journals	30000.00	15107.00	1500.00	16607.00
ii.	Equipment	400000.00	400000.00	--	400000.00
iii.	Contingency	45000.00	16474.00	29109.00	45583.00
iv.	Field Work/Travel (Give details in the proforma at Annexure-IV)	15000.00	8049.00	6951.00	15000.00
v.	Hiring Services	50000.00	18645.00	28992.00	47637.00
vi.	Chemicals & Glassware	100000.00	75080.00	16458.00	91538.00
vii.	Overhead	99000.00	99000.00	--	99000.00
viii.	Any other items (Please specify) Project fellowship	300000.00	76067.00	140000.00	216067.00
Total		**1039000.00**	**708422.00**	**223010.00**	**931432.00**
Remarks		--	UC and SOE sent on 9th August 2016	--	--

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c . Staff

Date of Appointment: 18th September 2015

Sr. No	Items	From	To	Amount Approved (Rs.)	Expenditure incurred (Rs.)	Remarks
1.	Honorarium to PI (Retired Teachers) @ Rs. 18,000/-p.m.	--	--	--	--	--
2.	**Project fellow: Non-GATE/Non-NET**- Rs.14,000/- p.m. for initial 2 years.	18th September 2015	31st March 2016	300000.00	76067.00	UC and SOE sent on 9th August 2016
		1st April 2016	1st January 2017	300000.00	140000.00	--
Total				**300000.00**	**216067.00**	--

1. It is certified that the appointment(s) have been made in accordance with the terms and conditions laid down by the Commission.
2. If as a result of check or audit objection some irregularly is noticed at later date, action will be taken to refund, adjust or regularize the objected amounts.
3. Payment @ revised rates shall be made with arrears on the availability of additional funds.
4. It is certified that the grant of Rs. 10,39,000/- (Rupees Ten Lakhs Thirty Nine Thousand Only) received from the University Grants Commission under the scheme of support for Major Research Project entitled 'Evidence Based Evaluation Of Medicinal Plants Used For Treating Polycystic Ovary Syndrome' vide UGC letter No. F. No. - 43-121/2014 (SR) dated 12th August 2015 out of which Rs. 9,31,432/- (Rupees Nine Lakhs Thirty One Thousand Four Hundred and Thirty Two Only) has been utilized for the purpose for which it was sanctioned and in accordance with the terms and conditions laid down by the University Grants Commission. The balance amount of Rs. 1,07,568/- remaining unspent (for the period from 1st July 2015 - 1st January 2017) will be utilized to meet the project objectives during the period (2nd January 2017 - 30th June 2018).

SIGNATURE OF THE PRINCIPAL INVESTIGATOR

PRINCIPAL INVESTIGATOR

SIGNATURE OF THE CO-INVESTIGATOR

RAMNARAIN RUIA COLLEGE 1937 MUMBAI

REGISTRAR/PRINCIPAL
(Seal)

Principal
Ramnarain Ruia College,
Matunga, Mumbai-400 019.

ANIL PANDHARINATH KELKAR Chartered Accountant

UNIVERSITY GRANTS COMMISSION

BAHADUR SHAH ZAFAR MARG

NEW DELHI – 110 002

STATEMENT OF EXPENDITURE INCURRED ON PROJECT FELLOW FOR THE YEAR 2015-2017

Name of the Principal Investigator: Dr. Sunita Shailajan

Name of the Junior Research Fellow: Ms. Suhina Bhosale

Sr. No.	Month	Date	Expenditure Incurred (Rs.)
1	Fellowship for the month of September 2015	18th September – 30th September 15	6067.00
2	Fellowship for the month of October 2015	1st October 15 – 31st October 15	14000.00
3	Fellowship for the month of November 2015	18th November 15 – 30th November 15	14000.00
4	Fellowship for the month of December 2015	18th December 15 – 31st December 15	14000.00
5	Fellowship for the month of January 2016	1st January – 31st January 16	14000.00
6	Fellowship for the month of February 2016	1st February – 29th February 16	14000.00
7	Fellowship for the month of March 2016	1st March – 31st March 16	14000.00
8	Fellowship for the month of April 2016	1st April – 30th April 16	14000.00
9	Fellowship for the month of May 2016	1st May – 31st May 16	14000.00
10	Fellowship for the month of June 2016	1st June – 30th June 16	14000.00
11	Fellowship for the month of July 2016	1st July – 31st July 16	14000.00
12	Fellowship for the month of August 2016	1st August – 31st August 16	14000.00

13	Fellowship for the month of September 2016	1st September – 30th September 16	14000.00
14	Fellowship for the month of October 2016	1st October – 31st October 16	14000.00
15	Fellowship for the month of November 2016	1st November – 30th November 16	14000.00
16	Fellowship for the month of December 2016	1st December – 31st December 16	14000.00
		Total	**216067.00**

Certified that the above expenditure is in accordance with the UGC norms for Major Research Projects.

SIGNATURE OF THE PRINCIPAL INVESTIGATOR

PRINCIPAL INVESTIGATOR

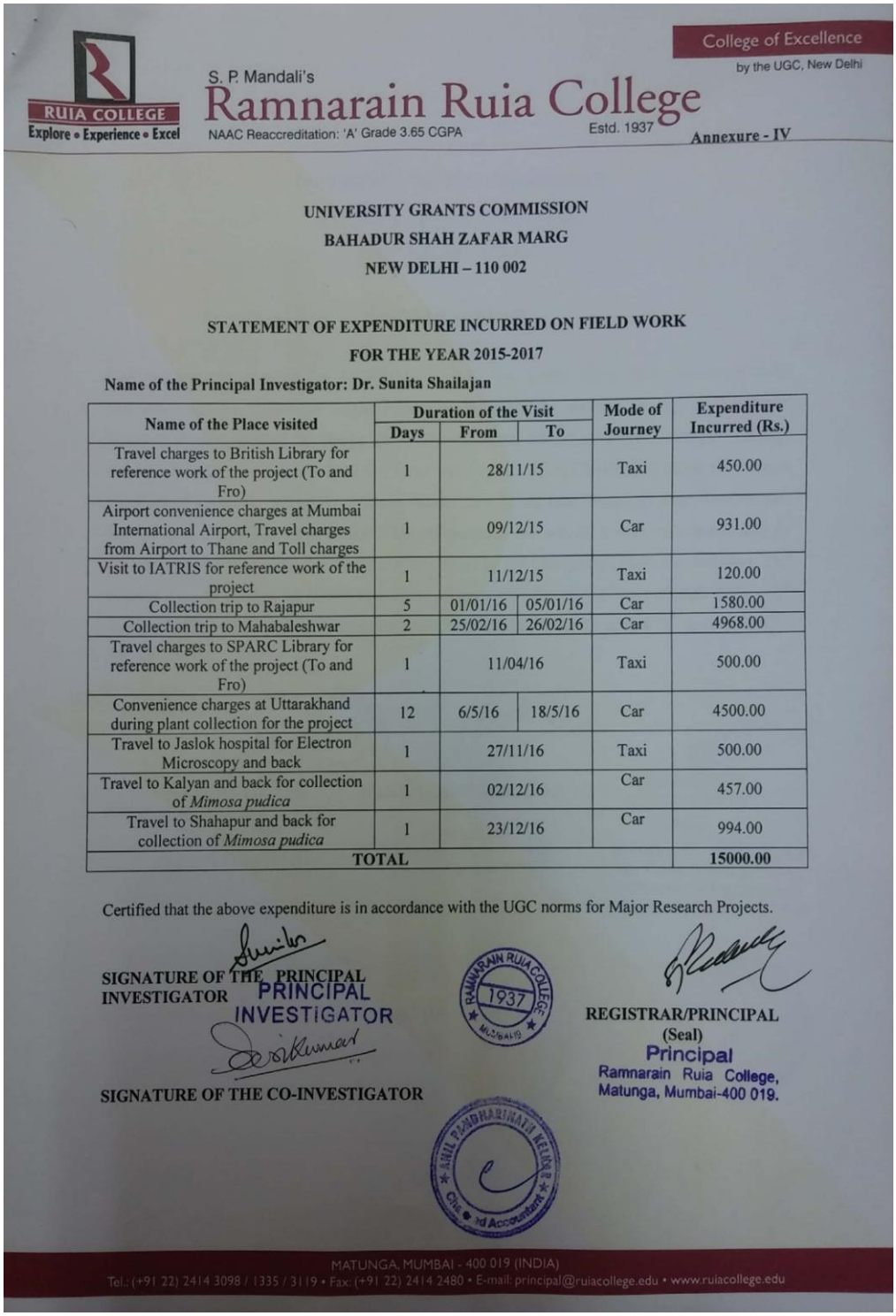
SIGNATURE OF THE CO-INVESTIGATOR

RAMNARAIN RUIA COLLEGE 1937 MUMBAI

REGISTRAR/PRINCIPAL
(Seal)
Principal
Ramnarain Ruia College.
Matunga, Mumbai-400 019.

ANIL PRABHAKARNATH KELKAR Chartered Accountant

Statement of Expenditure incurred on Field work for the year 2015-2017



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Annexure - IV

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

STATEMENT OF EXPENDITURE INCURRED ON FIELD WORK
FOR THE YEAR 2015-2017

Name of the Principal Investigator: Dr. Sunita Shailajan

Name of the Place visited	Duration of the Visit			Mode of Journey	Expenditure Incurred (Rs.)
	Days	From	To		
Travel charges to British Library for reference work of the project (To and Fro)	1	28/11/15		Taxi	450.00
Airport convenience charges at Mumbai International Airport, Travel charges from Airport to Thane and Toll charges	1	09/12/15		Car	931.00
Visit to IATRIS for reference work of the project	1	11/12/15		Taxi	120.00
Collection trip to Rajapur	5	01/01/16	05/01/16	Car	1580.00
Collection trip to Mahabaleshwar	2	25/02/16	26/02/16	Car	4968.00
Travel charges to SPARC Library for reference work of the project (To and Fro)	1	11/04/16		Taxi	500.00
Convenience charges at Uttarakhand during plant collection for the project	12	6/5/16	18/5/16	Car	4500.00
Travel to Jaslok hospital for Electron Microscopy and back	1	27/11/16		Taxi	500.00
Travel to Kalyan and back for collection of *Mimosa pudica*	1	02/12/16		Car	457.00
Travel to Shahapur and back for collection of *Mimosa pudica*	1	23/12/16		Car	994.00
TOTAL					**15000.00**

Certified that the above expenditure is in accordance with the UGC norms for Major Research Projects.

SIGNATURE OF THE PRINCIPAL INVESTIGATOR

PRINCIPAL INVESTIGATOR

SIGNATURE OF THE CO-INVESTIGATOR

RAMNARAIN RUIA COLLEGE 1937 MUMBAI-19

REGISTRAR/PRINCIPAL
(Seal)
Principal
Ramnarain Ruia College,
Matunga, Mumbai-400 019.

MATUNGA, MUMBAI - 400 019 (INDIA)
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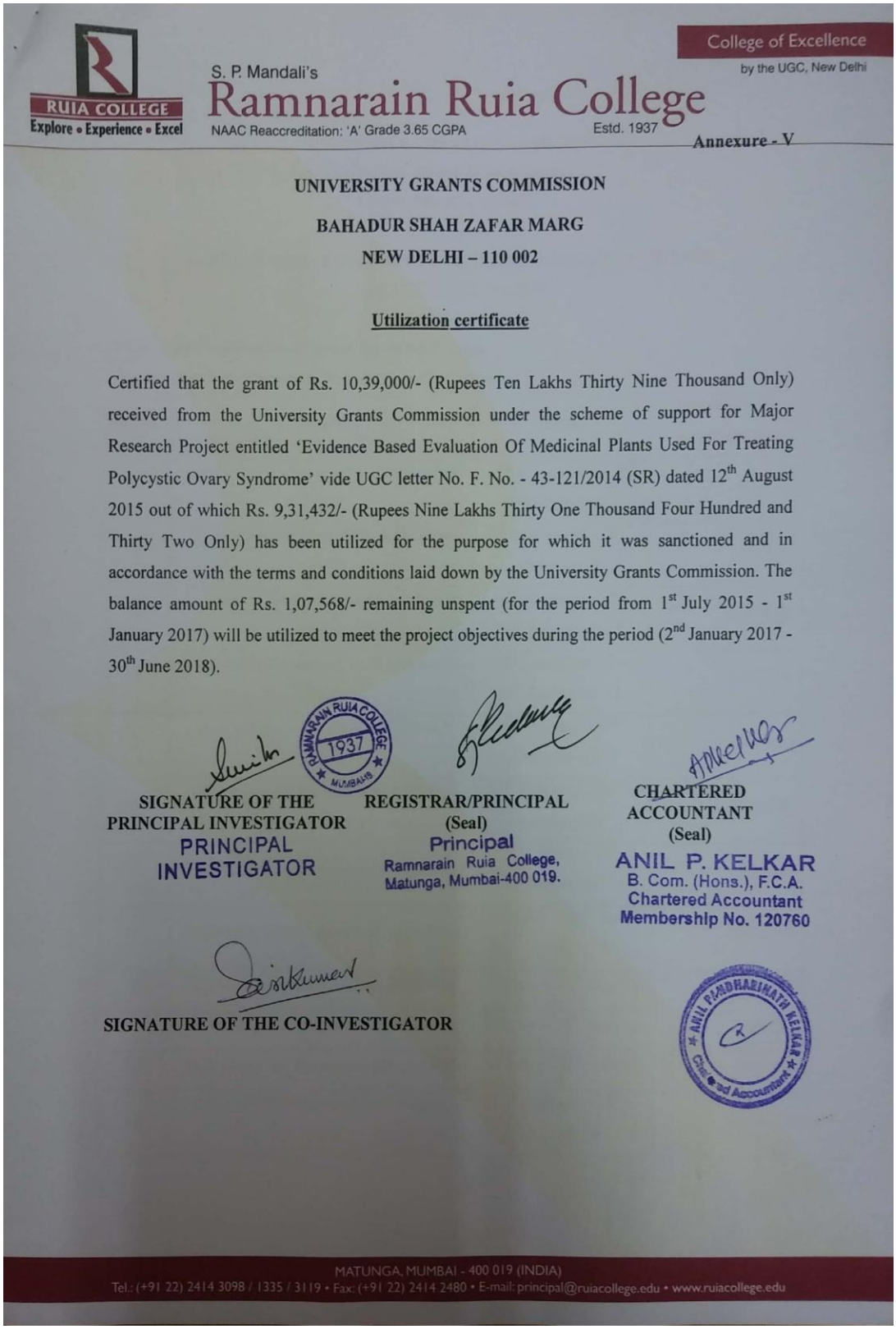
Utilization certificate from 1st April 2016-1st January 2017



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Annexure - V

UNIVERSITY GRANTS COMMISSION

BAHADUR SHAH ZAFAR MARG

NEW DELHI – 110 002

Utilization certificate

Certified that the grant of Rs. 10,39,000/- (Rupees Ten Lakhs Thirty Nine Thousand Only) received from the University Grants Commission under the scheme of support for Major Research Project entitled 'Evidence Based Evaluation Of Medicinal Plants Used For Treating Polycystic Ovary Syndrome' vide UGC letter No. F. No. - 43-121/2014 (SR) dated 12th August 2015 out of which Rs. 9,31,432/- (Rupees Nine Lakhs Thirty One Thousand Four Hundred and Thirty Two Only) has been utilized for the purpose for which it was sanctioned and in accordance with the terms and conditions laid down by the University Grants Commission. The balance amount of Rs. 1,07,568/- remaining unspent (for the period from 1st July 2015 - 1st January 2017) will be utilized to meet the project objectives during the period (2nd January 2017 - 30th June 2018).

RAMNARAIN RUIA COLLEGE 1937 MUMBAI

SIGNATURE OF THE PRINCIPAL INVESTIGATOR

PRINCIPAL INVESTIGATOR

REGISTRAR/PRINCIPAL (Seal)

Principal
Ramnarain Ruia College,
Matunga, Mumbai-400 019.

CHARTERED ACCOUNTANT (Seal)

ANIL P. KELKAR
B. Com. (Hons.), F.C.A.
Chartered Accountant
Membership No. 120760

SIGNATURE OF THE CO-INVESTIGATOR

ANIL PUNDHARINATH KELKAR Chartered Accountant

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Statement of Expenditure from 1st April 2016- 1st January 2017

Ramnarain Ruia College

Name of the Scheme: University Grants Commission (Major Research Project)
Sanctioning Authority: (F.No. -43-121/2014(SR) dated 12th August 2015

April 2016 - January 2017

Sr.no	Date	Code	Particulars	Recurring							Non recurring	Total Expenditure
				Project Fellowship	Chemicals & Consumables	Travel & field work	Contingency	Hiring services	Institutional overhead	Books & Journals	Equipments	Total
1	6/5/2016	B0037	Salary paid to Subina Bhosale for the month of March 2016	14000								14000
2	9/5/2016	C0155	Prof Tax (March 2016)									
3	6/5/2016	B0038	Salary paid to Subina Bhosale for the month of (April 2016)	14000								14000
4	9/5/2016	C0155	Prof Tax (April 2016)									
5	7/5/2016	C0145	Payment as per Voucher: Miscellaneous (Charges for purchase of stationery, pendrive, cleaning material, speed post)		839	500	1462	1000		1500		5301
6	13/5/2016	B0111	Cheque paid to: Anutek services (Repair of Plunger seal and diaphragm for HPLC pump)				15188					15188
7	8/6/2016	C0298	Payment as per Voucher: Miscellaneous (Charges for plant collection) -Uttarakhand		7035	4500	8498	3200				23233
8	17/6/2016	B0226	Salary paid to Subina Bhosale for the month of May 2016	14000								14000
9	7/7/2016	B0285	Salary paid to Subina Bhosale for the month of June 2016	14000								14000
10	26/7/2016	C0645	Payment as per Voucher: Miscellaneous (Charges for Calibration of HPTLC spotter, scanner, photodocumentation unit, monopan balance, pipettes)					5050				5050
11	22/8/2016	C0814	Payment as per Voucher: Miscellaneous (Speed post charges, stationary)		2433		2760					5193
12	22/8/2016	C0822	Payment as per Voucher: Animal study (Approval of protocols and monitoring of the perusion of liver tissue)		44			5200				5244
13	30/8/2016	B0491	Salary paid to Subina Bhosale for the month of July 2016	14000								14000
14	12/9/2016	B0620	Salary paid to Subina Bhosale for the month of August 2016	14000								14000
15	17/10/2016	B0776	Salary paid to Subina Bhosale for the month of September 2016	14000								14000
16	18/10/2016	C1178	Payment as per Voucher: Annual report preparation		336			5100				5436
17	27/10/2016	B0863	Salary paid to Subina Bhosale for the month of October 2016	14000								14000
18	22/12/2016	B1187	Salary paid to Subina Bhosale for the month of November 2016	14000								14000
19	Jan-17	B1282	Salary paid to Subina Bhosale for the month of December 2016	14000								14000
20	Jan-17	C1733	Payment as per voucher: Miscellaneous (sample for analysis,stationary)		3635	500	1201					5336
21	Jan-17	C1734	Payment as per Voucher: Hiring services					5200				5200
22	Jan-17	B1349	Cheque paid to: Yugandhara Patil (Technical expertise)					2045				2045
23	Jan-17	B1350	Cheque paid to: Mayuresh Joshi (Evaluation of safety profile)		2136			2197				4333
24	Jan-17	C1817	Payment as per voucher: Travel charges for plant collection			1451						1451
	TOTAL			140000	16458	6951	29109	28992	0	1500	0	223010

Certified that the amount of Rs. 223010.00 (Rs. Two Lakhs Twenty Three Thousand and Ten Only) has been spent for the purpose for which the grant was sanctioned and in accordance with the terms and conditions laid down in F. No. -43-121/2014 (SR) dated 12th August 2015. The balance amount Rs. 1,07,568/- remaining unspent (for the period from 1st July 2015 - 1st January 2017) will be utilized to meet the project objectives during the period (2nd January - 30th June 2018).

Place: Mumbai
Date:

Dr. Sudha Shailajan
Principal Investigator

PRINCIPAL INVESTIGATOR

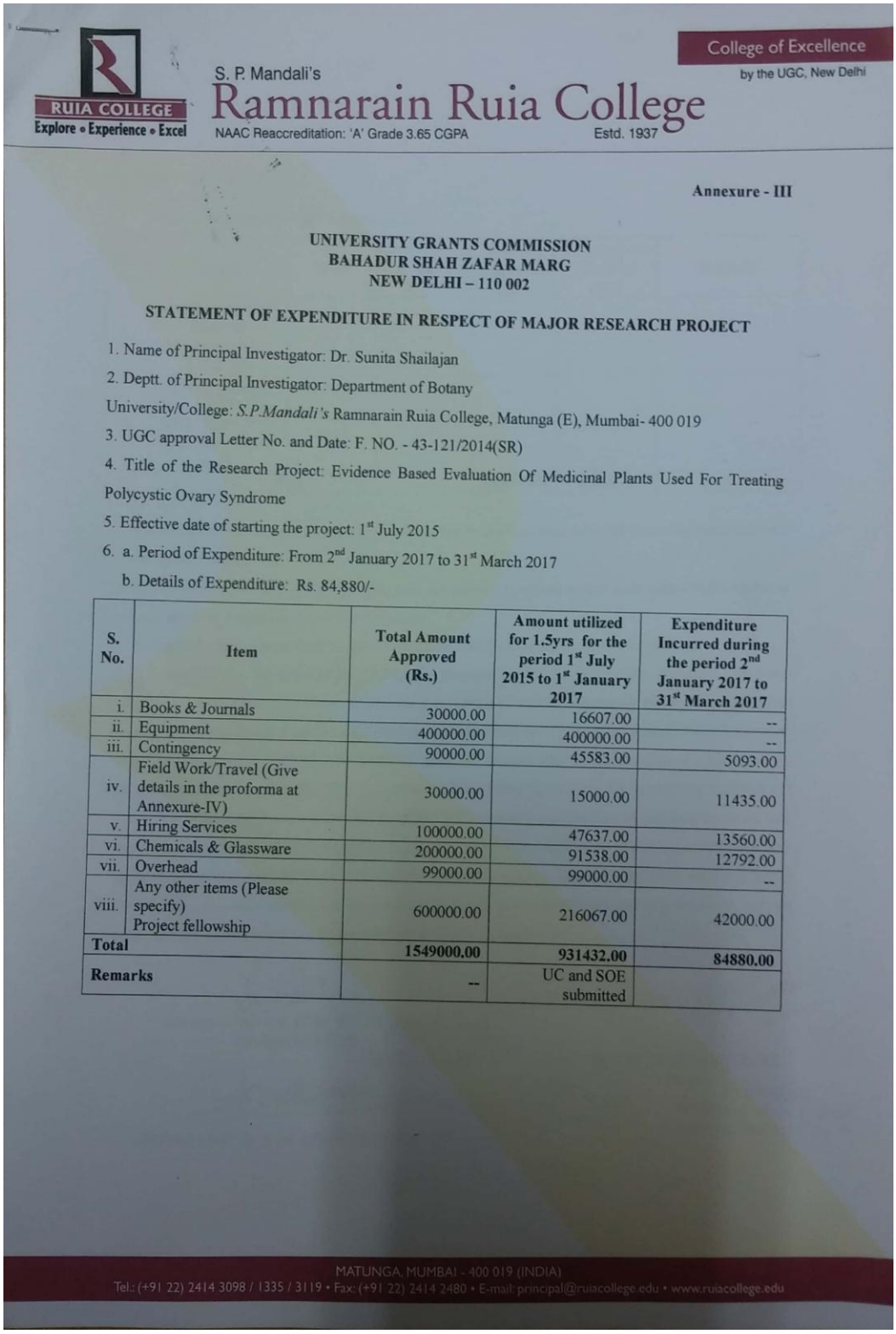
Prof (Dr.) Subas Pednekar
Principal

Principal
Ramnarain Ruia College,
Matunga, Mumbai-400 019.

Chartered Accountant

ANIL P. KELKAR
B. Com. (Hons.), F.C.A.
Chartered Accountant
Membership No. 120760

Statement of Expenditure from 2nd January 2017-31st March 2018



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Annexure - III

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

STATEMENT OF EXPENDITURE IN RESPECT OF MAJOR RESEARCH PROJECT

1. Name of Principal Investigator: Dr. Sunita Shailajan
2. Deptt. of Principal Investigator: Department of Botany
University/College: *S.P.Mandali's* Ramnarain Ruia College, Matunga (E), Mumbai- 400 019
3. UGC approval Letter No. and Date: F. NO. - 43-121/2014(SR)
4. Title of the Research Project: Evidence Based Evaluation Of Medicinal Plants Used For Treating Polycystic Ovary Syndrome
5. Effective date of starting the project: 1st July 2015
6. a. Period of Expenditure: From 2nd January 2017 to 31st March 2017
   b. Details of Expenditure: Rs. 84,880/-

S. No.	Item	Total Amount Approved (Rs.)	Amount utilized for 1.5yrs for the period 1st July 2015 to 1st January 2017	Expenditure Incurred during the period 2nd January 2017 to 31st March 2017
i.	Books & Journals	30000.00	16607.00	--
ii.	Equipment	400000.00	400000.00	--
iii.	Contingency	90000.00	45583.00	5093.00
iv.	Field Work/Travel (Give details in the proforma at Annexure-IV)	30000.00	15000.00	11435.00
v.	Hiring Services	100000.00	47637.00	13560.00
vi.	Chemicals & Glassware	200000.00	91538.00	12792.00
vii.	Overhead	99000.00	99000.00	--
viii.	Any other items (Please specify) Project fellowship	600000.00	216067.00	42000.00
Total		**1549000.00**	**931432.00**	**84880.00**
Remarks		--	UC and SOE submitted	

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c. Staff

Date of Appointment: 18th September 2015

Sr. No	Items	From	To	Amount Approved (Rs.)	Expenditure incurred (Rs.)	Remarks
1.	Honorarium to PI (Retired Teachers) @ Rs. 18,000/-p.m.	--	--	--	--	--
2.	**Project fellow:** Non-GATE/Non-NET- Rs.14,000/- p.m. for initial 2 years.	2nd January 2017	31st March 2017	300000.00	42000.00	
Total				**300000.00**	**42000.00**	--

1. It is certified that the appointment(s) have been made in accordance with the terms and conditions laid down by the Commission.
2. If as a result of check or audit objection some irregularly is noticed at later date, action will be taken to refund, adjust or regularize the objected amounts.
3. Payment @ revised rates shall be made with arrears on the availability of additional funds.
4. It is certified that the grant of Rs. 10,39,000/- (Rupees Ten Lakhs Thirty Nine Thousand Only) was received from the University Grants Commission under the scheme of support for Major Research Project entitled 'Evidence Based Evaluation Of Medicinal Plants Used For Treating Polycystic Ovary Syndrome' vide UGC letter No. F. No. - 43-121/2014 (SR) dated 12th August 2015 for the duration of 1.5 years of the project. From the sanction, Rs. 9,31,432/- (Rupees Nine Lakhs Thirty One Thousand Four Hundred and Thirty Two Only) has been utilized for the purpose for which it was sanctioned and in accordance with the terms and conditions laid down by the University Grants Commission and the UC and SOE have been submitted. The balance amount of Rs. 1,07,568/- remaining unspent has been carried forward and from it Rs. 84,880/- has been utilized for the purpose for which it was sanctioned and in accordance with the terms and conditions laid down by the University Grants Commission.

SIGNATURE OF THE PRINCIPAL INVESTIGATOR

PRINCIPAL INVESTIGATOR

SIGNATURE OF THE CO-INVESTIGATOR

REGISTRAR/PRINCIPAL
(Seal)

RAMNARAIN RUIA COLLEGE 1937 MUMBAI-19.

Principal
Ramnarain Ruia College,
Matunga, Mumbai-400 019.

UNIVERSITY GRANTS COMMISSION

BAHADUR SHAH ZAFAR MARG

NEW DELHI – 110 002

STATEMENT OF EXPENDITURE INCURRED ON PROJECT FELLOW

FOR THE YEAR JAN 2017-MAR 2017

Name of the Principal Investigator: Dr. Sunita Shailajan

Name of the Junior Research Fellow: Ms. Suhina Bhosale

Sr. No.	Month	Date	Expenditure Incurred (Rs.)
1	Fellowship for the month of January 2017	1st January 17 – 31st January 17	14000.00
2	Fellowship for the month of February 2017	1st February 17 – 28th February 17	14000.00
3	Fellowship for the month of March 2017	1st March 17 – 31st March 17	14000.00
		Total	**42000.00**

Certified that the above expenditure is in accordance with the UGC norms for Major Research Projects.

SIGNATURE OF THE PRINCIPAL INVESTIGATOR

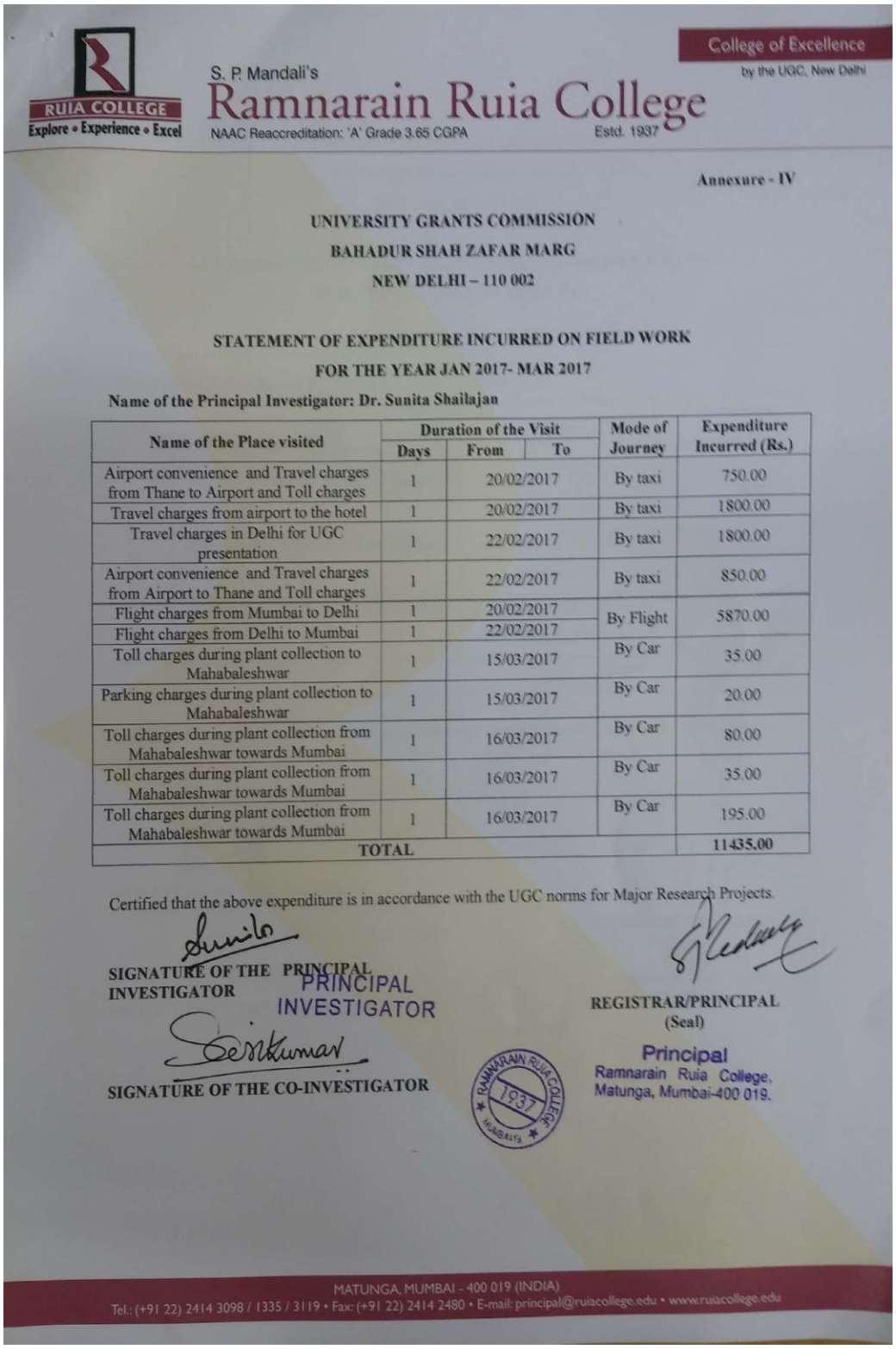
PRINCIPAL INVESTIGATOR

SIGNATURE OF THE CO-INVESTIGATOR

RAMNARAIN RUIA COLLEGE 1937 MUMBAI-19

REGISTRAR/PRINCIPAL
(Seal)
Principal
Ramnarain Ruia College,
Matunga, Mumbai-400 019.

Statement of Expenditure incurred on Field work from 2nd January 2017- 31st March 2017



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Annexure - IV

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

STATEMENT OF EXPENDITURE INCURRED ON FIELD WORK
FOR THE YEAR JAN 2017- MAR 2017

Name of the Principal Investigator: Dr. Sunita Shailajan

Name of the Place visited	Duration of the Visit			Mode of Journey	Expenditure Incurred (Rs.)
	Days	From	To		
Airport convenience and Travel charges from Thane to Airport and Toll charges	1	20/02/2017		By taxi	750.00
Travel charges from airport to the hotel	1	20/02/2017		By taxi	1800.00
Travel charges in Delhi for UGC presentation	1	22/02/2017		By taxi	1800.00
Airport convenience and Travel charges from Airport to Thane and Toll charges	1	22/02/2017		By taxi	850.00
Flight charges from Mumbai to Delhi	1	20/02/2017		By Flight	5870.00
Flight charges from Delhi to Mumbai	1	22/02/2017			
Toll charges during plant collection to Mahabaleshwar	1	15/03/2017		By Car	35.00
Parking charges during plant collection to Mahabaleshwar	1	15/03/2017		By Car	20.00
Toll charges during plant collection from Mahabaleshwar towards Mumbai	1	16/03/2017		By Car	80.00
Toll charges during plant collection from Mahabaleshwar towards Mumbai	1	16/03/2017		By Car	35.00
Toll charges during plant collection from Mahabaleshwar towards Mumbai	1	16/03/2017		By Car	195.00
TOTAL					11435.00

Certified that the above expenditure is in accordance with the UGC norms for Major Research Projects.

SIGNATURE OF THE PRINCIPAL INVESTIGATOR

PRINCIPAL INVESTIGATOR

SIGNATURE OF THE CO-INVESTIGATOR

REGISTRAR/PRINCIPAL
(Seal)

Principal
Ramnarain Ruia College,
Matunga, Mumbai-400 019.

RAMNARAIN RUIA COLLEGE 1937 MUMBAI-19

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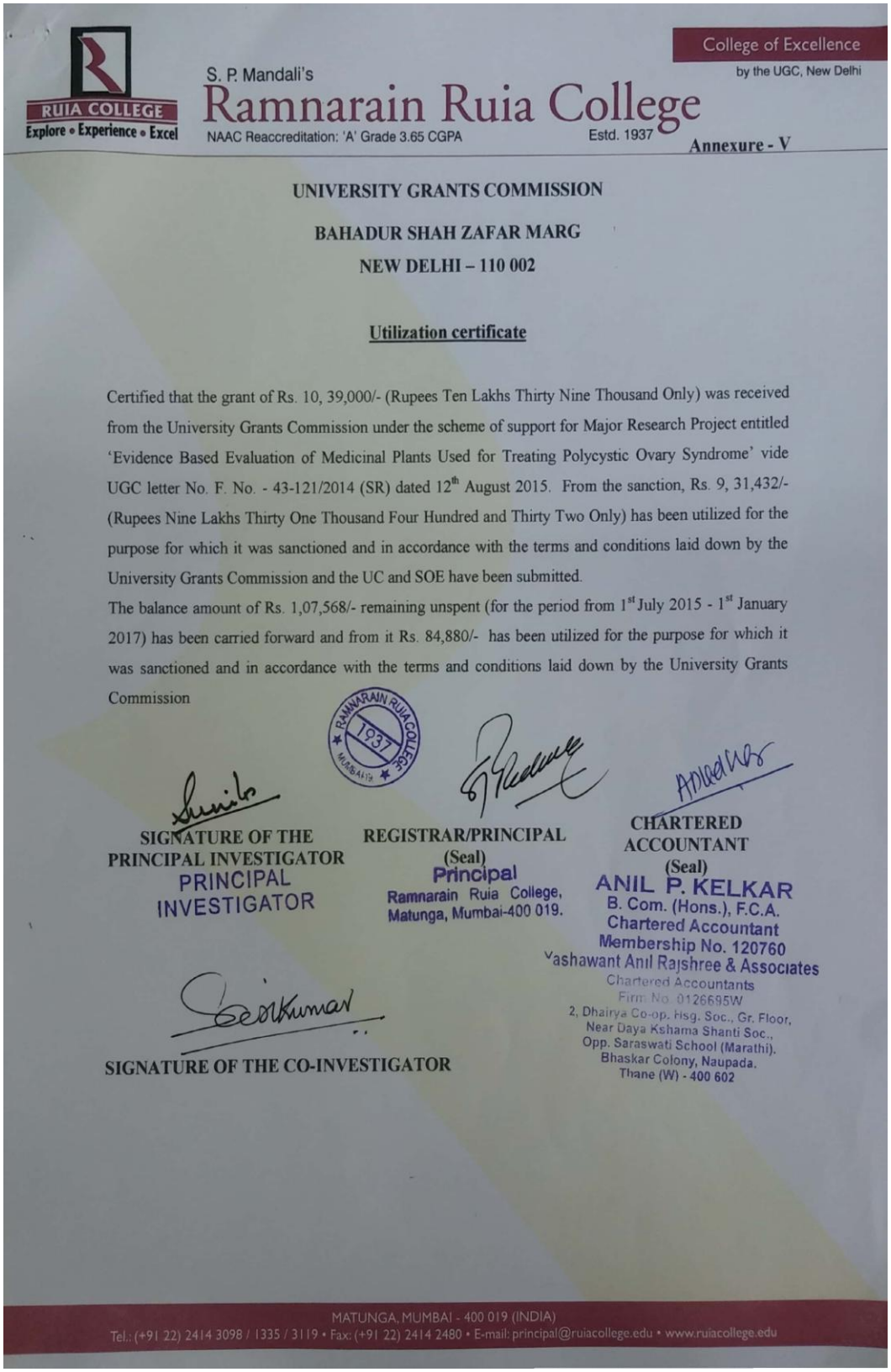
Utilization certificate from 2nd January 2017- 31st March 2017



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Annexure - V

UNIVERSITY GRANTS COMMISSION

BAHADUR SHAH ZAFAR MARG

NEW DELHI – 110 002

Utilization certificate

Certified that the grant of Rs. 10, 39,000/- (Rupees Ten Lakhs Thirty Nine Thousand Only) was received from the University Grants Commission under the scheme of support for Major Research Project entitled 'Evidence Based Evaluation of Medicinal Plants Used for Treating Polycystic Ovary Syndrome' vide UGC letter No. F. No. - 43-121/2014 (SR) dated 12th August 2015. From the sanction, Rs. 9, 31,432/- (Rupees Nine Lakhs Thirty One Thousand Four Hundred and Thirty Two Only) has been utilized for the purpose for which it was sanctioned and in accordance with the terms and conditions laid down by the University Grants Commission and the UC and SOE have been submitted.

The balance amount of Rs. 1,07,568/- remaining unspent (for the period from 1st July 2015 - 1st January 2017) has been carried forward and from it Rs. 84,880/- has been utilized for the purpose for which it was sanctioned and in accordance with the terms and conditions laid down by the University Grants Commission

RAMNARAIN RUIA COLLEGE 1937 MUMBAI

SIGNATURE OF THE PRINCIPAL INVESTIGATOR

PRINCIPAL INVESTIGATOR

REGISTRAR/PRINCIPAL
(Seal)

Principal
Ramnarain Ruia College,
Matunga, Mumbai-400 019.

CHARTERED ACCOUNTANT
(Seal)

ANIL P. KELKAR
B. Com. (Hons.), F.C.A.
Chartered Accountant
Membership No. 120760
Vashawant Anil Rajshree & Associates
Chartered Accountants
Firm No. 0126695W
2, Dhairya Co-op. Hsg. Soc., Gr. Floor,
Near Daya Kshama Shanti Soc.,
Opp. Saraswati School (Marathi).
Bhaskar Colony, Naupada.
Thane (W) - 400 602

SIGNATURE OF THE CO-INVESTIGATOR

MATUNGA, MUMBAI - 400 019 (INDIA)
Tel.: (+91 22) 2414 3098 / 1335 / 3119 • Fax: (+91 22) 2414 2480 • E-mail: principal@ruiacollege.edu • www.ruiacollege.edu

Statement of expenditure from 2nd January 2017- 31st March 2017

Ramnarain Ruia College

Name of the Scheme: University Grants Commission (Major Research Project)
Sanctioning Authority: (F.No. -43-121/2014(SR) dated 12th August 2015

January 2017 - March 2017

Sr. no	Date	Code	Particulars	Recurring							Non recurring	Total Expenditure
				Fellowship	Chemicals & Consumables	Travel & field work	Contingency	Hiring services	Institutional overhead	Books & Journals	Equipments	Total
1	8/2/2017	B1417	Salary paid to Subina Bhosale for the month of January 2017	14000								14000
2	8/2/2017	C1947	Payment as per voucher: Hiring service (charges for extract preparation of Mimosa pudica and study the safety profile of extracts)					5160				5160
3	8/2/2017	C1948	Payment as per voucher: contingency (charges for purchase of stationary, pendrive and plant powder containers)		356		5063					5419
4	14/3/2017	C2267	Payment as per voucher: Mid term evaluation meet of UGC (Travel charges accomodation and refreshment charges)		2794	2550						5344
5	20/3/2017	C2317	Payment as per voucher: Miscellaneous (Refreshment charges,travel charges)		2473	2650						5123
6	21/3/2017	B1609	Salary paid to Subina Bhosale for the month of February 2017	14000								14000
7	21/3/2017	B1611	Cheque paid to V. V Laps travel (Travel to Delhi for UGC Midterm evaluation meet)			5870						5870
8	29/3/2017	C2447	Payment as per voucher: Miscellaneous (Refreshment charges, accomodation charges, collection of samples)		7169	365	30	1200				8764
9	31/03/2017	B1784	Salary paid to Subina Bhosale for the month of March 2017	14000								14000
10	31/03/2017	B1913	Cheque paid to Sangita Shetty (Bills for testing of rat plasma samples)					7200				7200
	TOTAL			42000	12792	11435	5093	13560	0	0	0	84880

Certified that the amount of Rs. 84880.00 (Rs. Eighty Four Thousand Eight Hundred and Eighty Only) has been spent for the purpose for which the grant was sanctioned and in accordance with the terms and conditions laid down in F. No. - 43-121/2014 (SR) dated 12th August 2015.

Place: Mumbai
Date:

Dr. Sunita Shailajan
Principal Investigator
PRINCIPAL INVESTIGATOR

RAMNARAIN RUIA COLLEGE 1937 MUMBAI

Prof (Dr.) Suhas Pednekar
Principal
Principal
Ramnarain Ruia College,
Mahim, Mumbai-400 019.

Chartered Accountant
ANIL P. KELKAR
B. Com. (Hons.), F.C.A.
Chartered Accountant
Membership No. 120760
Yashawant Anil Rajshree & Associates
Chartered Accountants
Firm No. 125955W
2, Dhanraj Mahal Chs. Ltd., Gr. Floor,
Near Vidya Mandir Shala Soc.,
Opp. Saraswati School (Marathi)

Statement of Expenditure from 1st April 2017-31st March 2018

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Annexure - III

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

STATEMENT OF EXPENDITURE IN RESPECT OF MAJOR RESEARCH PROJECT

1. Name of Principal Investigator: Dr. Sunita Shailajan
2. Deptt. of Principal Investigator: Department of Botany
University/College : *S.P.Mandali's* Ramnarain Ruia College, Matunga (E), Mumbai- 400 019
3. UGC approval Letter No. and Date: F. NO. - 43-121/2014(SR)
4. Title of the Research Project: Evidence Based Evaluation Of Medicinal Plants Used For Treating Polycystic Ovary Syndrome
5. Effective date of starting the project: 1st July 2015
6. a. Period of Expenditure: From 1st April 2017 to 31st March 2018
   b. Details of Expenditure: Rs. 2,97,469/-

S.No	Item	Total Amount Approved (Rs.)	Grant sanctioned (II nd installment)	Expenditure Incurred(Rs) 1st April 2017 to 31st March 2018
i.	Books & Journals	30000.00	00.00	00.00
ii.	Equipment	400000.00	00.00	00.00
iii.	Contingency	90000.00	36000.00	16939.00
iv.	Field Work/Travel (Give details in the proforma at Annexure-IV).	30000.00	12000.00	3625.00
v.	Hiring Services	100000.00	40000.00	31800.00
vi.	Chemicals & Glassware	200000.00	80000.00	59105.00
vii.	Overhead	99000.00	--	00.00
viii.	Any other items (Please specify) Project fellowship	600000.00	138240.00	186000.00
Total		**1549000.00**	**306240.00**	**297469.00**

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c . Staff

Date of Appointment: 18th Sept

S.No	Items	From	To	Amount Approved (Rs.)	Expenditure incurred (Rs.)
1.	Honorarium to PI (Retired Teachers) @ Rs. 18,000/-p.m.	--	--	--	--
2.	**Project fellow:** **NET/GATE qualified**-Rs. 16,000/- p.m.for initial 2 years and Rs. 18,000/- p.m. for the third year. **Non-GATE/Non-NET-** Rs.14,000/- p.m. for initial 2 years and Rs. 16,000/- p.m. for the third year.	1st April 2016	31st March 2017	138240.00	186000.00

1. It is certified that the appointment(s) have been made in accordance with the terms and conditions laid down by the Commission.
2. If as a result of check or audit objection some irregularly is noticed at later date, action will be taken to refund, adjust or regularize the objected amounts.
3. Payment @ revised rates shall be made with arrears on the availability of additional funds.
4. It is certified that the grant of Rs. 3,06,240.00 (Rupees Three Lakhs Six Thousand Two Hundred and Forty Only) received from the University Grants Commission under the scheme of support for Major Research Project entitled 'Evidence Based Evaluation Of Medicinal Plants Used For Treating Polycystic Ovary Syndrome' vide UGC letter No. F. No. - 43-121/2014 (SR) dated 12th August 2015 out of which Rs. 2,97,469.00 (Rupees Two Lakhs Ninety Seven Thousand Four Hundred and Sixty Nine Only) has been utilized for the purpose for which it was sanctioned and in accordance with the terms and conditions laid down by the University Grants Commission. The balance amount Rs. 8,771.00 (Rupees Eight Thousand Seven Hundred and Seventy One only) remaining unspent for the period from 1st April 2017 - 31st March 2018 will be utilized to meet the project objectives during the period (1st April 2018 - 30th June 2018).

SIGNATURE OF THE PRINCIPAL INVESTIGATOR

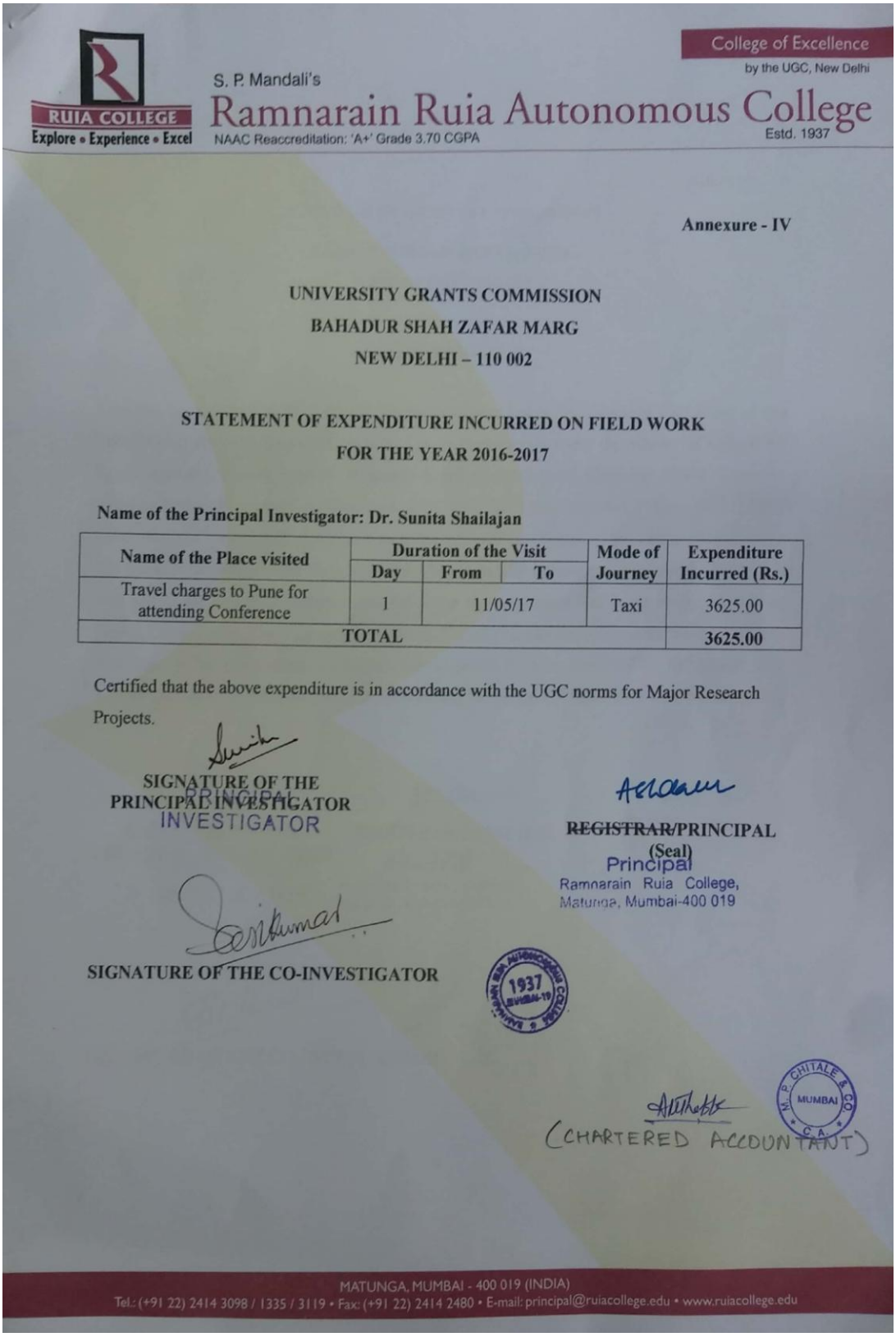
PRINCIPAL INVESTIGATOR

SIGNATURE OF THE CO-INVESTIGATOR

~~REGISTRAR~~/PRINCIPAL
(Seal)
Principal
Ramnarain Ruia College,
Matunga, Mumbai-400 019

(CHARTERED ACCOUNTANT)

Statement of Expenditure incurred on Field work from 1st April 2017-31st March 2018



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NAAC Reaccreditation: 'A+' Grade 3.70 CGPA
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Annexure - IV

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

STATEMENT OF EXPENDITURE INCURRED ON FIELD WORK
FOR THE YEAR 2016-2017

Name of the Principal Investigator: Dr. Sunita Shailajan

Name of the Place visited	Duration of the Visit			Mode of Journey	Expenditure Incurred (Rs.)
	Day	From	To		
Travel charges to Pune for attending Conference	1	11/05/17		Taxi	3625.00
TOTAL					3625.00

Certified that the above expenditure is in accordance with the UGC norms for Major Research Projects.

SIGNATURE OF THE
PRINCIPAL INVESTIGATOR
PRINCIPAL
INVESTIGATOR

~~REGISTRAR~~/PRINCIPAL
(Seal)
Principal
Ramnarain Ruia College,
Matunga, Mumbai-400 019

SIGNATURE OF THE CO-INVESTIGATOR

(CHARTERED ACCOUNTANT)

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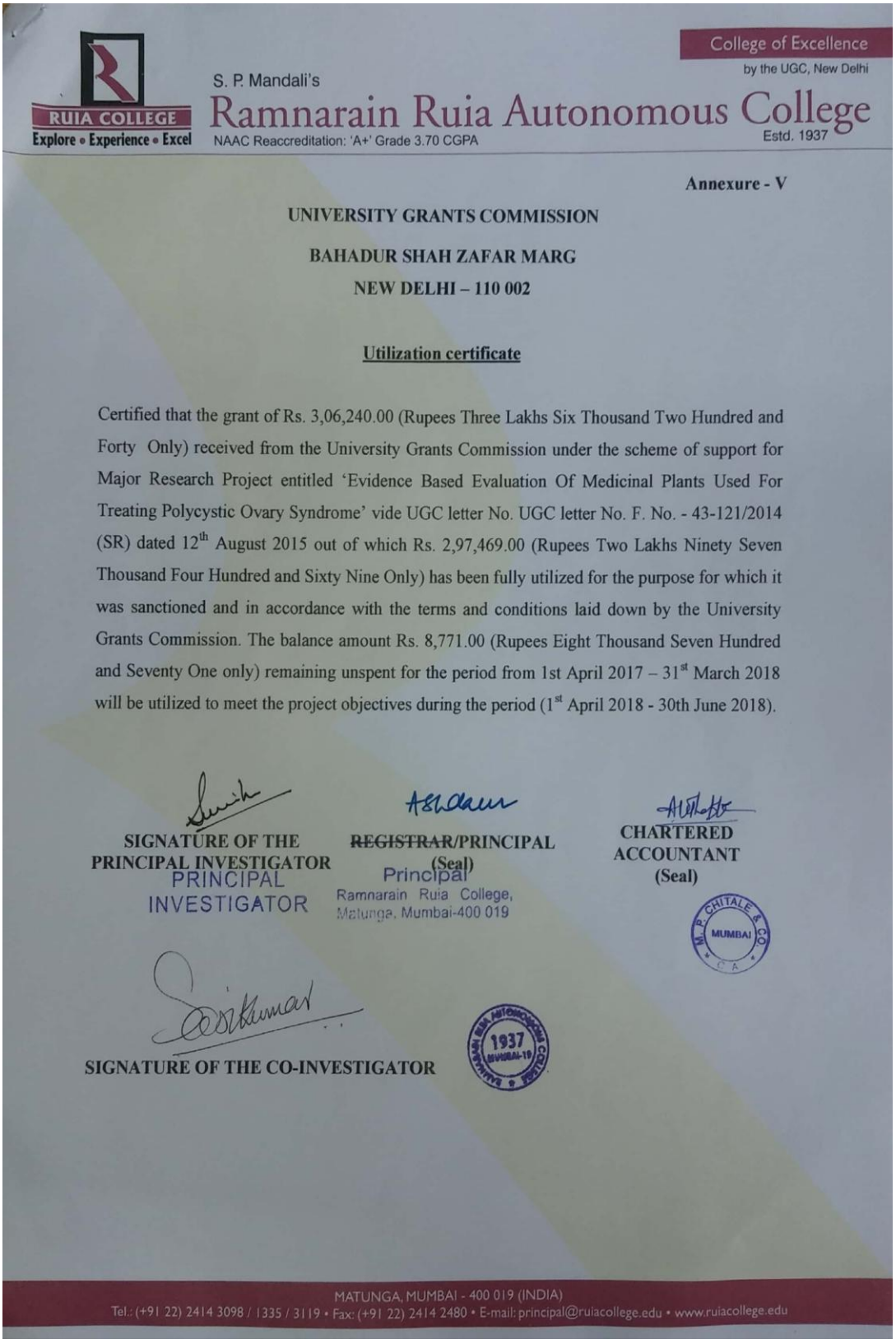
Tel.: (+91 22) 2414 3098 / 1335 / 3119 • Fax: (+91 22) 2414 2480 • E-mail: principal@ruiacollege.edu • www.ruiacollege.edu

Utilization certificate from 1st April 2017- 31st March 2018



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NAAC Reaccreditation: 'A+' Grade 3.70 CGPA
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Annexure - V

UNIVERSITY GRANTS COMMISSION

BAHADUR SHAH ZAFAR MARG

NEW DELHI – 110 002

Utilization certificate

Certified that the grant of Rs. 3,06,240.00 (Rupees Three Lakhs Six Thousand Two Hundred and Forty Only) received from the University Grants Commission under the scheme of support for Major Research Project entitled 'Evidence Based Evaluation Of Medicinal Plants Used For Treating Polycystic Ovary Syndrome' vide UGC letter No. UGC letter No. F. No. - 43-121/2014 (SR) dated 12th August 2015 out of which Rs. 2,97,469.00 (Rupees Two Lakhs Ninety Seven Thousand Four Hundred and Sixty Nine Only) has been fully utilized for the purpose for which it was sanctioned and in accordance with the terms and conditions laid down by the University Grants Commission. The balance amount Rs. 8,771.00 (Rupees Eight Thousand Seven Hundred and Seventy One only) remaining unspent for the period from 1st April 2017 – 31st March 2018 will be utilized to meet the project objectives during the period (1st April 2018 - 30th June 2018).

SIGNATURE OF THE PRINCIPAL INVESTIGATOR

PRINCIPAL
INVESTIGATOR

~~REGISTRAR~~/PRINCIPAL (Seal)

Principal
Ramnarain Ruia College,
Matunga, Mumbai-400 019

CHARTERED ACCOUNTANT (Seal)

M. P. CHITALE & CO.
MUMBAI

SIGNATURE OF THE CO-INVESTIGATOR

1937
MUMBAI-19

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Statement of Expenditure from 1st April 2017- 31st March 2018

Ramnarain Ruia Autonomous College

Name of the Scheme: University Grants Commission (Major Research Project)

Sanctioning Authority: (F.No. -43-121/2014(SR) dated 12th August 2015

April 2017 - March 2018

Sr.n	Date	Code	Particulars	Recurring							Non recurring			Total Expenditure
				Project Fellowship	Chemicals & Consumables	Travel & field work	Contingency	Hiring services	Institutional overhead	Books & Journals	Equipments	Without GST	GST	Total
1	9/5/2017	BP00112	Cheque paid to: Sangeeta Shetty (Bill for testing of rat plasma)					6000				6000		6000
2	17/5/2017	CP00180	Payment as per Voucher: Miscellaneous expenses (travel charges from Mumbai to Pune, Registration charges)		2890	3625						6515		6515
3	17/5/2017	CP00181	Payment as per Voucher: Extract Preparation (extract preparation, purchase of sample for analysis, xerox charges(		6795		1454					8249		8249
4	30/5/2017	BP00117	Payment of telephone bil				551					551		551
5	30/5/2017	BP00118	Salary paid to Subina Bhosale for the month of April 2017	14000								14000		14000
6	9/6/2017	BP00169	Payment of telephone bil				600					600		600
7	27/6/2017	BP00235	Salary paid to Subina Bhosale for the month of May 2017	14000								14000		14000
8	12/7/2017	BP00321	Salary paid to Subina Bhosale for the month of June 2017	14000								14000		14000
9	1/8/2017	CP00548	cash in hand (Hiring service)					4550				4550		4550
10	8/8/2017	CP00588/ CP00589	Payment as per voucher: Miscellaneous expenses (stationary, extract preparation, purchase of sample for analysis)		2680		1537	1100				5317		5317
11	16/9/2017	BP00598	Salary paid to Subina Bhosale for the month of July and August 2017	32000								32000		32000
12	22/9/2017	CP00777	Payment as per voucher: Miscellaneous expenses (extract preparation, dosing of rats for pharmacokinetic studies, withdrawal of blood)					5470				5470		5470
13	3/10/2017	CP00812	Payment as per voucher miscellaneous expenses (extract preparation, extraction of marker from plasma)					5300				5300		5300
14	6/10/2017	BP00696	Salary paid to Subina Bhosale for the month of Sept 2017	16000								16000		16000
15	25/10/2017	CP00939	Payment as per voucher hiring services (Interpretation of histology slides of different extracts of plants)					6180				6180		6180
16	10/11/2017	BP00845	Salary paid to Subina Bhosale for the month of Oct 2017	16000								16000		16000
17	13/11/2017	BP00851	cheque paid to Arihant Enterprise		3534							2994.9	539.1	3534
18	14/11/2017	CP01016	payment as per voucher miscellaneous expenses (preparation of extracts, refreshment charges, stationary charges)		5268		1050					6318		6318
19	14/11/2017	CP01017	Payment as per voucher Travel charges for plant collection (extract preparation of Mimosa pudica in different solvents)		6200							6200		6200
20	14/11/2017	CP01018	Payment as per Voucher consumables (safety evaluation of ethanolic extract of Mimosa pudica)		5210							5210		5210

Ramnarain Ruia Autonomous College

Name of the Scheme: University Grants Commission (Major Research Project)

Sanctioning Authority: (F.No. -43-121/2014(SR) dated 12th August 2015

April 2017 - March 2018

Sr.no	Date	Code	Particulars	Recurring							Non recurring			Total Expenditure
				Project Fellowship	Chemicals & Consumables	Travel & field work	Contingency	Hiring services	Institutional overhead	Books & Journals	Equipments	Without GST	GST	Total
21	30/11/2017	CP01141	Payment as per voucher Plant collection for preparation of extract (Interpretation of LM slides for project work, sample for analysis, refreshment charges)		5054		120					5121.12	52.88	5174
22	11/12/2017	BP00999	Salary paid to Suhina Bhosale for the month of Nov 2017	16000								16000		16000
23	8/1/2018	BP01130	salary paid to Suhina Bhosale for the month of December 2017	16000								16000		16000
24	15/2/2018	BP01324	salary paid to Suhina Bhosale for the month of January 2018	16000								16000		16000
25	17/2/2018	BP01364	cheque paid to prototype (Purchase of microsoft office software for project work)				4250					4250		4250
26	13/3/2018	BP01543	salary paid to Suhina Bhosale for the month of February 2018	16000								16000		16000
27	19/3/2018	CP01949	Payment as per voucher Consumables (registration charges for international conference, stationary charges, refreshment charges)		3840		1516					5356		5356
28	19/3/2018	CP01950	Payment as per voucher Consumables for project work (callibration of HPLC syringe and pump, refreshment charges, extraction of marker from plasma)		1720		200	3200				5120		5120
29	19/3/2018	CP01951	Payment as per voucher Consumables for project work (extraction of phytoconstituents from plant, purchase of stationary, purchase of sample for analysis)		5109		154					5263		5263
30	19/3/2018	CP01952	Payment as per voucher Miscellaneous expenses (purchase of stationary, extract preparation, registration charges for poster competition)		4835		1337					6172		6172
31	19/3/2018	CP01953	payment as per voucher: Miscellaneous expenses (membership charges, extract preparation, refreshment charges)		5970							5970		5970
32	22/3/2018	CP01994-CP01997	Cash in hand Contingency (laptop cable, IC unit repair, laptop adaptor, printer carriage assembly)				4170					4170		4170
33	31/3/2018	BP01834	salary paid to Suhina Bhosale for the month of March 2018	16000								16000		16000
	TOTAL			186000	59105	3625	16939	31800	0	0	0	296877.02	591.98	297469

Certified that the amount of Rs. 297469.00 (Rs. Two Lakhs ninety Seven Thousand Four Hundred and Sixty Nine Only) has been spent for the purpose for which the grant was sanctioned and in accordance with the terms and conditions laid down in F. No. - 43-121/2014 (SR) dated 12th August 2015.

29 JUN 2018

Place: Mumbai
Date:

Dr. Sunita Shailaja
Principal Investigator

PRINCIPAL INVESTIGATOR

Principal
Ramnarain Ruia College,
Matunga, Mumbai-400 019

Chartered Accountant

Details of Conferences attended

Gmail sunita shailajan <sunitashailajan@gmail.com>

ABSTRACTS- Ramnarain Ruia College, Matunga, Mumbai

Department Students' Welfare <deptstudentswelfare@gmail.com> Tue, Feb 14, 2017 at 12:19 PM
To: sunita shailajan <sunitashailajan@gmail.com>

Greetings from University of Mumbai

On behalf of Technical Committee of 8th Indian Youth Science Congress, I am pleased to inform you that your abstract has been accepted for 'Poster Presentation'. You are requested to complete the Process of Registration on Thursday, February 16, 2017 at 9:00 a.m. at Registration Desk, Green Technology Building, Vidyanagari (Kalina Campus), University of Mumbai.

You are also requested to attend all the sessions of the 8th Indian Youth Science Congress scheduled from February 16, 2017 to February 18, 2017.

I would like to extend my congratulations to you and I look forward to welcoming you to the 8th Indian Youth Science Congress.

Note: The outstation students (Especially from Ratnagiri and Sindhudurga Districts) will get the information of their accommodation at the Registration Desk.

For further Assistance (If required), Please Contact to

Dr. Uttam Kendre (Chairman, Accommodation Committee), 9892001500

Dr. Chandrakant Puri (Chairman, Registration Committee), 9819056444

[Quoted text hidden]

--

Dr. Sunil S. Patil, M.Sc., M.Phil., Ph.D.
DIRECTOR,
DEPARTMENT STUDENTS' WELFARE,
UNIVERSITY OF MUMBAI,
'B' ROAD, CHURCHGATE, MUMBAI-400020
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sunita shailajan <sunitashailajan@gmail.com>

Updated list of Oral and Poster Presentations for SFEC-2017 with codes

SFEC2017 Bardoli <sfec2017bardoli@utu.ac.in> Fri, Feb 17, 2017 at 7:10 PM
Cc: Ramar Krishnamurthy <krishnashanti@gmail.com>, vijayalakshmi.ghosh@utu.ac.in, Nallanchakravarthula Srivathsa <n.srivathsa@utu.ac.in>
Bcc: sunitashailajan@gmail.com

Dear Delegates,

Greetings from SFEC-2017 Local Organizing Committee.

Please find attached updated list of Oral and Poster Presentations with codes.

The file is updated and re-numbered w.r.t. to some abstracts after withdrawal. Kindly notice the changes.

Please consider the **Codes in the attached file as final for Oral or Poster presentation during SFEC-2017.**

Looking forward to meet you in SFEC-2017.

Thank you.

Best regards,
Dr Vijayalakshmi Ghosh
--

Organizing Secretary, SFEC-2017

C. G. Bhakta Institute of Biotechnology

Uka Tarsadia University

Bardoli, Gujarat

http://utu.ac.in/sfec2017bardoli

2 attachments

List of Oral & Poster Presentations with Codes.pdf
652K

Program Schedule.pdf
526K

58.	SFEC-17/PP/058B	**Brijesh K. Yadav**[1], Sangeeta[1], Ritesh K. Jaiswal[2], Sunita P and Achuit K. Singh[1,3]	Mungbean Yellow Mosaic India Virusin Association with Tomato Leaf Curl Beta Satellite Causing Disease in Soybean Crop
59.	SFEC-17/PP/059B	**Kalyani Kale**, Bhavana Hirudkar, Rasika Mankar and Shekhar Waikar.	*Syzygium cumini*: A Review
60.	SFEC-17/PP/060B	Sunita Shailajan[1], Sasikumar Menon[2], **Suhina Bhosale**[1] and Mayuresh Joshi[1]	Comparative HPTLC and HPLC Estimation of Betulinic Acid from *Mimosa pudica* Collected from Various Geographical Regions of India
61.	SFEC-17/PP/061B	**Sonal Sharma** and Neeta Shrivastava	Development of DNA Barcodes for Molecular Identification of Plant Species Equated With The Common Name "Shankhpushpi"
62.	SFEC-17/PP/062B	**P.G.Bhavishi**, K.Anand Babu And K.Chitra	Phytochemical and HPTLC Studies of Ethanolic Extract of The Bark of *Syzigium cumini*
63.	SFEC-17/PP/063B	**Pal Santosh**., Itankar P.R. and Prasad S.K.	A Comparative Study of Physicochemical and Phytochemical Evaluation of Ethanolic and Aqueous Extract of *Caesalpinia crista* Seeds
64.	SFEC-17/PP/064B	**Ritika B.Yadav**	Pseudocereals: Nutritional Quality and Potential Health Benefits
65.	SFEC-17/PP/065B	**P. L. Bharati** and Ankush D. Jadhav	Ethnobotanical Survey and Documentation of Medicinal Plants Used by Nicobari Tribes of Andaman and Nicobar Islands
66.	SFEC-17/PP/066B	**Puja Patel** and Srivathsa Nallanchakravarthula	Evaluation of Antibacterial Activity of *Boerhaavia diffusa* Leaves Extract on Gram-Positive Multi Drug Resistant Bacteria Isolated from Soil
67.	SFEC-17/PP/067B	**Prajapati Bhumi Kirit Kumar**, Acharya Varsha Kumari, Sangha Bijekar and Naga Rathna Supriya	Significant Events in Phytochemistry
68.	SFEC-17/PP/068B	**Panchal Dixita**, Nagarathna Supriya and Sangha Bijekar	Endophytes: An Alternative Source of Medicinal Plants
69.	SFEC-17/PP/069B	**Smita Parekh**, Naga Rathna Supriya and Sangha Bijekar	Unveiling The Importance of Botanist In Forensics
70.	SFEC-17/PP/070B	**Ayushi Patel**, Monika Goswami, Jeel Shah, Shraddha Saha Shreya Desai, Swetal Patel and Rajshekhar Ingalhalli	Chia (*Salvia hispanica* L.) – A Multifaceted Plant with Great Potential
71.	SFEC-17/PP/071B	**Swati Patel**, Ashutosh Pathak and Aruna Joshi	Effect of Cytokinins, Explant Age and its Orientation on Shoot Regeneration in *Tylophora indica* (Burm. F.) Merrill. Leaf Explant
72.	SFEC-17/PP/072B	**Mariyn Patel**	Review on Research Studies of *Caryota urens*
73.	SFEC-17/PP/073B	**Pooja Patel**[1], R. Krishnamurthy[2]and Ashok Shah[3]	Isolation and Screening of Phosphate Solubilising Yeasts from the Flower of *Madhuca longifolia*
74.	SFEC-17/PP/074B	**Divya V. Sangode**, Prakash R. Itankar and Satyendra K. Prasad	A Comparative Phytochemical Standardization of Two Solanum Species i.e. *Solanum nigrum* and *Solanum indicum*
75.	SFEC-17/PP/075B	**Swetal Patel**, Shraddha Saha, Shreya Desai and R. Ingalhalli	Efficacy of *Anisomelis indica* Leaves as Mosquito Repellent
76.	SFEC-17/PP/076B	**Soumen Samanta**, Prakash R. Itankar and Satyendra Kumar Prasad	A Comparative Phytochemical Study of Wild and Cultivated *Solanum indicum* Roots
77.	SFEC-17/PP/077B	**Rathod Priyanka**, Patel Kilvisha, Sangha Bijekar and Naga Rathna Supriya	A Critical Review on Dragon Fruit

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Status of abstract submitted to 4th Bharatiya Vigyan Sammelan 2015

BVS three <bvs2015ms@gmail.com> Thu, Jan 8, 2015 at 3:40 PM
To: Sartaj Bhat <sartajbhat88@gmail.com>, Subhasmit Sinha <sinhasubhasmit77@gmail.com>, parasu raman <parasuvenkataraman@gmail.com>, KUNAL MONDAL <kunalmondal1985@yahoo.com>, Goldy Shah <goldyshah@gmail.com>, AJAY THAKUR <dr_asthakur@rediffmail.com>, Vivek Talwar <vivek_ptu@yahoo.in>, ranjan ghosh <ranjanghoshaum@yahoo.co.in>, Tambor Lyngdoh <tamborlyngdoh70@gmail.com>, Pooja Agrawal <dr.poojagrawal@gmail.com>, Raghavendra Naik <raghavchaos@gmail.com>, Vyas Dhamodaran <vyas2sayv@gmail.com>, jaison joseph <jaisonjosephp@gmail.com>, Adarsh Pal Vig <adarshpalvig@yahoo.co.in>, Braja Sundar Mishra <brajasundarmishra@gmail.com>, sapanasasane@yahoo.com, pks_ageg@iari.res.in, URAIN NAMAH <urainnamah@yahoo.com>, Shyam Verma <nrcshyam@gmail.com>, rajpootns@rediffmail.com, DURGA DUTT Ozha <ddozha@gmail.com>, sameer patil <patilfriends@gmail.com>, Sachin Narawdiya <snarwadiya@gmail.com>, Amita Watkar <amitawatkar2@gmail.com>, drgovindpandey@rediffmail.com, njoshigeo@gmail.com, Riddhi rajyaguru <riddhi7474@gmail.com>, Sukh Dev Sharma <sukhdevsharma40@gmail.com>, Manoj Kumar <manojdasbhu@gmail.com>, koushik_iari@rediffmail.com, nahiriyas@yahoo.com, YOGESH DANDEKAR <dandekar.yogesh@gmail.com>, "dr jamuna.n m pillai" <drjamunantc@gmail.com>, kiran bais <kiransinghbais@gmail.com>, nandit jadvani <nandit.jadvani@gmail.com>, vijay kumar vaidya <vkvaidya2503@gmail.com>, arundhatijp@gmail.com, Debarati Bhaduri <debarati.ssiari@gmail.com>, Sandhya Babhalgaonkar <babhalgaonkars.ccoew@gmail.com>, kedar chimote <kedarchimote@gmail.com>, sayali shende <sayali.shende17@gmail.com>, sadhnaashish2003@yahoo.com, DrSanjay Tiwari

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Dear Author,

This is to inform that your abstract submitted to 4th Bharatiya Vigyan Sammelan (BVS), has been accepted for presentation. To know the mode of presentation, either oral or poster, you may look at the website of BVS www.bvs2015.in under the heading "received abstracts". Also please see the heading "templates" for details regarding the number of slides to be presented for oral and the details of poster presentation.

Those who wish to avail accommodation may make the payment for registration and accommodation on or before 20th January 2015. Any queries regarding accommodation, please contact +919423061614 or e-mail deep1917@yahoo.co.in, ikpai@unigoa.ac.in . It is not possible to get accommodation for onsite registration. Any queries on transportation, please contact +919326139104 or e-mail. manohar_pednekar@yahoo.com

See you soon in Goa

Best regards

BVS Secretariat

Gmail sunita shailajan <sunitashailajan@gmail.com>

Final Revised notification about Research Paper Acceptance and Presentation

Green Earth <greenearthbnb@gmail.com> Tue, Dec 19, 2017 at 9:17 PM
To: Rathod L R <lrathod78@yahoo.com>, Jhuma Sutradhar <jsdhar1987@gmail.com>, lokhoabba@gmail.com, Moumita Das <moumitataxonomy@gmail.com>, A P Das <apdas.nbu@gmail.com>, minakshi gurav <minakshi.gurav@ruparel.edu>, Ashwini Deshpande <ashwini_d1@yahoo.com>, Aashka Rajput <aashkaanil98@gmail.com>, Shweta Valanju <shwetarv06@gmail.com>, Mangesh Jamble <mangeshjamble@gmail.com>, bindu gopalkrishnan <bindu_phd@rediffmail.com>, soniya shetty <shetty.soniya2@gmail.com>, prajakta mathur <sweet_praj2003@yahoo.co.in>, Chetana shetty <shettychetana72@gmail.com>, Aruna Rai <aru_r17@hotmail.com>, Nusrat Shaikh <nusratshaikh1212@gmail.com>, Shweta Chitnis <schitnis69@gmail.com>, ketakee avinash Deodhar <ketbot@gmail.com>, Urmila Kumavat <urmi.22@rediffmail.com>, paras jadhav <ppjadhav2107@gmail.com>, Snehal Phirke <snehal26@gmail.com>, Moitreyee Saha <sahamoitreyee@gmail.com>, Suchandra Dutta <suchandra.dutta@gmail.com>, Pooja Gupta <poojaog84@gmail.com>, Priyanka Verma <88v.priyanka@gmail.com>, Vaibhav Jawalekar <vaibhavjawalekar@gmail.com>, neha aggarwal <neha_264840@yahoo.co.in>, Zahera Momin <momin.zahera@gmail.com>, shivani kakkar <kakkar.shivani89@gmail.com>, plant lovers <rojabotany1@gmail.com>, Seema <seemanarkhede12@rediffmail.com>, "Dr. Rajni" <rajni.tewari@gmail.com>, drsasikumarmenon@ruiacollege.edu, sunita shailajan <sunitashailajan@gmail.com>, Neelam Parab <nparab10@gmail.com>, Satish Malode <satishmalode17@gmail.com>, "Dr V M Jamdhade ji" <drvinodj@gmail.com>, maninder dhaliwal <dmkaur@gmail.com>, Shraddha Pandey <shraddhapandey220@gmail.com>, Ashwini Gadi <ashugadi1994@gmail.com>, Arvind Kumar Shukla <arvindkumarshukla10@gmail.com>, daxesh patel <dk.patel001@yahoo.com>, bhalchandra mandlekar <bhalchandra.k.mandlekar@gmail.com>, ashish koli <ashish.koli.thane@gmail.com>, sangita meshram <sangita.meshram@rediffmail.com>, gunwanti negi <gunwanti89@gmail.com>, antagoswamigiri@gmail.com, Shirish Bapat <shirishdbapat@gmail.com>, ranjan tiwari <ranjan23691@gmail.com>, "Cerebro ." <vsp144@live.com>, harsha padwal <harshapadwal@gmail.com>, Sanjay Gaikwad <drsanjaydgaikwad@gmail.com>, kiran kumar Eripogu <kirankumareripogu@gmail.com>, Sukhada Jambale <jambalesm.dc@gmail.com>, Pallavi Chandwaskar <pchandwaskar@vpmthane.org>, Dona Joseph <dj28172@gmail.com>, Vini Somaiya <vini.somaiya1994@gmail.com>, Shruti Handa <spk2shruti@gmail.com>, Ashish Singh <ashish.singh@keva.co.in>, HARSHAL BHOIR <harshal.bhoir@vpmthane.org>, Laxmikant Deshpande <amartd@godrej.com>, Hemant Karkhanis <hvk@godrej.com>, sanjivbp@godrej.com, tmj@godrej.com, Papiya Deb <papiyadeb@gmail.com>

Dear Authors,

It gives immense pleasure to receive huge response in form of Full Length Research Papers from all authors. Green Earth team appreciate your valuable contribution towards compilation of Proceeding of 'Multidisciplinary International Conference on Green Earth: A panoramic view', scheduled on 12th -13th January 2018.

Please find attachment of **List of Accepted Full Length Research Papers.**

You are requested to check the final **Paper Code** allotted to your Research Paper and **Mode of Presentation (Oral / Poster)** mentioned. Kindly note that Mode of presentation of your research paper has been finalized by **Reviewer Panel.**

An additional document of **Instructions to Presenters** is also attached for your information. Every presenter author is requested to go through this document carefully and follow all instructions correctly.

You are also informed that registrations of all authors mentioned in given list have been confirmed by Organizing Committee. As the Certificate of your name will be made ready prior to Conference, you are

24	POSTER	215	Study of Quantitative Phytochemistry and Antioxidant Potential of Two Allied Species Of Pink Cassias ------ Nilima Pandey, Pranita Ambre, Snehal Bhangale and Urmila Kumavat
25	POSTER	216	Physicochemical, Phytochemical, HPTLC and GC-MS Analysis of *Lagenaria Siceraria* (Molina) Standl. and *Luffa Cylindrica* (L.) M. Roem. Vegetable Peel Waste -------- Shivani Kakkar and Meeta Bhot
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29	POSTER	220	Standardisation of Quality Parameters For "*Avala supari*" Using High Performance Liquid Chromatography -------- Pooja Jaiswar, Asma Pathan, Prajakta Walkunde, Mangesh Bharti, Bhimrao Kawale, Mandar Mhatre and Sasikumar Menon
30	POSTER	221	Comparative Evaluation of Curcumin In Marketed Topical Formulations Using High Performance Liquid Chromatography ------ Aishwarya Wadekar, Snehal Sangle, Yamuna Aihole, Ashish Khedekar, Mandar Mhatre and Sasikumar Menon
31	POSTER	222	High Performance Liquid Chromatographic Evaluation of Food Supplements Containing Beta Carotene ------ Dhananjay Chavan, Mayur Mane, Sayali Valavate, Kavita Dhumak, Mandar Mhatre and Sasikumar Menon
32	POSTER	223	Marker Based Standardization of An Ayurvedic Plant: *Mimosa pudica* L. ------- Sunita Shailajan, Suhina Bhosale, Mayuresh Joshi and Sasikumar Menon
33	POSTER	224	Modern Approach Towards Standardization of A Traditional Ayurvedic Formulation *Pushyanuga Churna*: A Need of The Hour ------ Sunita Shailajan, Swati Singh and Yugandhara Patil
34	POSTER	225	Comparative Evaluation of Kaempferol and Genistein From Three Species Of *Flemingia*: A Medicinal Plant ------- Sunita Shailajan, Reena Mascarenhas and Prachi Bagkar
35	ORAL	226	Use Of Bioactive Markers For Quality Evaluation of *Mustakarishta*: An Ayurvedic Formulation ------- Sabina Thareja, Sunita Shailajan, Sasikumar Menon, Mayuri Utekar and Bhavesh Tiwari
36	POSTER	227	Cultivation, Phytochemicals And Applications of *Sesbania bispinosa* (Jacq.) W. F. Wight ------- Neelam Parab and Sharda Vaidya
37	POSTER	228	Phytochemical Screening And Thin Layer Chromatography of Crude Extract of *Sauromatum venosum* (Ait.) Schott. Tuber ------- M. U. Ghurde and Malode S.N.

Publication Details

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sunita shailajan <sunitashailajan@gmail.com>

Decision Letter to Authors - Acceptance - (JAPS-2017-01-035)

Journal of Applied Pharmaceutical Science <japs@ejmanager.com> Thu, Feb 9, 2017 at 10:40 PM
Reply-To: "japs@ejmanager.com" <japs@ejmanager.com>
To: sunitashailajan@gmail.com

Dear Dr. Sunita Shailajan,

I am pleased to inform you that your manuscript titled as "Application of modern RP-HPLC technique for the quantitation of betulinic acid from traditional drug Symplocos Racemosa Roxb." (Manuscript Number: JAPS-2017-01-035) was accepted for publication in the Journal of Applied Pharmaceutical Science. You are required to submit article processing charges Rs. 2500/- INR within next three days of receiving this mail through Banking/Paypal. Payment details are as follows;

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